ÁLLATTENYÉSZTÉS

és TAKARMÁNYOZÁS

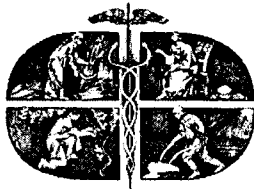### LEHETŐSÉGEK ÉS KIHÍVÁSOK A XXI. SZ. ÁLLATTENYÉSZTÉSÉBEN

OPPORTUNITIES AND CHALLENGES FOR THE ANIMAL INDUSTRY IN THE 21ST CENTURY

GYÖNGYÖS, 2003.

KÜLÖNSZÁM / SUPPLEMENT

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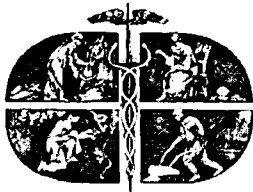IOWA STATE UNIVERSITY, AMES (USA)
M. E. ENSMINGER ENDOWMENT, AMES (USA)

OPPORTUNITIES AND CHALLENGES FOR THE ANIMAL INDUSTRY IN THE 21ST CENTURY

International Conference

Gyöngyös
October 15–17, 2003

**SZENT ISTVÁN EGYETEM
GAZDÁLKODÁSI ÉS MEZŐGAZDASÁGI FŐISKOLAI KAR
GYÖNGYÖS**

**IOWAI ÁLLAMI EGYETEM, AMES (USA)
M. E. ENSMINGER ALAPÍTVÁNY, AMES (USA)**

LEHETŐSÉGEK ÉS KIHÍVÁSOK A XXI. SZ. ÁLLATTENYÉSZTÉSÉBEN

Nemzetközi Konferencia

**Gyöngyös
2003. október 15–17.**

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TARTALOM

PREFACE

The Ensminger International Schools have had a rich tradition for more than 50 years. Dr. and Mrs. Ensminger conducted 71 Ensminger Schools in countries around the world. This is the first Ensminger School in central Europe.

Iowa State University's Animal Science Department received a wonderful gift from Marion Eugene Ensminger and Audrey Ensminger to continue the International Schools in cooperation with outstanding animal science programs throughout the world. We are proud to be a partner for the Hungary School with the College of Economics and Agriculture, Department of Animal Breeding, Saint Stephens University, Gyöngyös.

Professor Ensminger established the Agriservices Foundation, a non-profit foundation, serving world agriculture in the area of world food, hunger and malnutrition. In 1964, Dr. Ensminger developed the International Stockman's School in the United States in addition to the Schools in other countries. Professor Ensminger was an outstanding teacher and researcher at Washington State University before he moved to Clovis, California to become a full-time author of animal science books and President of the Agriservices Foundation. He published 22 outstanding animal science books.

The College of Agriculture at Iowa State University has had very successful cooperative programs with the College of Economics and Agriculture, Gyöngyös. During discussions of the successful cooperative programs, it was pointed out that animal agriculture in Hungary and other European countries had some significant challenges and special opportunities as we start the new century. The best way to overcome the challenges and take advantage of the opportunities is to have a good science foundation on which to obtain the correct answers and direction. Therefore, the Ensminger School for Hungary was established to help provide a stronger scientific foundation. Scientists from the leading animal research and educational institutions in Hungary formed a program committee and established the topics for the Ensminger School. The committee developed outstanding topics, and established scientists were invited to present the papers for the Ensminger School.

The Hungary School will feature 1) global problems facing animal agriculture and the impact on Hungary and European animal agriculture, 2) biotechnology and molecular genetics for the animal industry, and 3) food safety and nutrition for animal agriculture.

The papers in the proceedings for the Ensminger School were not peer reviewed. Therefore, the contents of the papers are the responsibility of the authors and not the journal.

We want to thank all of the individuals who contributed to the Ensminger School in Hungary, especially the speakers for their outstanding papers and the Planning Committee for all of their contributions to the planning of the Ensminger School.

David G. Topel

ELŐSZÓ

A 2003. október 15–17 között Gyöngyösön sorra kerülő „Lehetőségek és kihívások a XXI. sz. állattenyésztésében" c. rendezvény-sorozat előadásainak rövidített változatát tartalmazó kiadványt, az Állattenyésztés és Takarmányozás különszámát tartja kezében az Olvasó.

Az előadássorozat az Iowai Állami Egyetem, az Ensminger Alapítvány és a Szent István Egyetem Gyöngyösi Gazdálkodási és Mezőgazdasági Főiskolai Kara közös szervezésében kerül megrendezésre.

Az Ensminger Alapítvány által a világ különböző országaiban szervezett tanácskozások (Ensminger International Schools) nagy hagyománnyal rendelkeznek. A több mint 50 éves múltra visszatekintő konzultatív jellegű rendezvényeket Dr. Ensminger professzor és felesége kezdeményezte és vezette. Eddig 71 tanácskozás megrendezésére került sor.

Az utóbbi időben, az Iowai Állami Egyetem Állattenyésztéstani Tanszéke mellett működő Ensminger Alapítvány vette át az „iskolák" szervezését, miután Marion Eugene Ensminger és neje, Audray Ensminger által ráruházott jogként, tovább vezetheti a „Nemzetközi Ensminger Iskolát". A kialakult gyakorlatnak megfelelően, a világ különböző pontjain működő, kiemelkedő szakmai munkát végző intézményekkel karöltve kerül sor az állattenyésztés tudománya és a gyakorlat aktuális kérdéseit napirendre tűző tanácskozásokra, ahol rendszerint az adott régió és a világ állattenyésztése szempontjából meghatározó témák előadására és megvitatására kerül sor.

A rendezvények anyagi, és szellemi bázisát megteremtő, az állattenyésztő szakma fejlődése iránt rendkívüli elkötelezettségű Ensminger házaspárról tudni kell, hogy kezdetben egy non-profit szervezetet hoztak létre, az Agroservice Alapítványt, annak érdekében, hogy segítsék a világ elmaradott régióiban élők élelmiszer-ellátását. Ensminger professzor 1964-ben egy újabb szervezettel, a Nemzetközi Stockman Iskolával gyarapította az állattenyésztés fejlesztését elősegítő szervezeteket.

Ensminger professzor a Washingtoni Állami Egyetemen oktatott, de többek között tagja volt az Orosz Mezőgazdasági Tudományos Akadémiának. Kiváló tanár és kutató volt, számos könyv- és kézikönyv szerzője valamint több szabadalom tulajdonosa. Kiterjedt oktató munkája mellett, 22 kitűnő szakkönyvvel gazdagította tudományterületünket. Elnöke volt többek között az Agroservice Fundation-nak is.

Az Iowai Állami Egyetem, dr. D. Topel professzor dékáni tevékenysége idején, gyümölcsöző, kölcsönös előnyökön alapuló kapcsolatot alakított ki a Gyöngyösi Főiskolával. Az együttműködés keretében oktató- és hallgatócserére, közös kutatási programok végzésére, oktatási anyagok és tankönyvek cseréjére, doktori, valamint (az Iowai Egyetemen) MSc képzési lehetőség biztosítására került sor.

Az elmúlt évben, Topel professzor indítványát elfogadva, a két intézmény szellemi és anyagi támogatásával kezdtük meg az első európai „Ensminger School" szervezését Magyarországon. A Gyöngyösi Főiskolán folyó oktató- és kutatómunka jelentős elismerésének tekinthető, hogy az Intézmény társrendezője lehet ennek a hazai állattenyésztés sikereit is elismerő, a tudományterület további fejlődését meghatározó rendezvénynek.

A konferencia célkitűzésének megfogalmazása során folytatott tárgyaláson megállapítottuk, hogy az új évszázad küszöbén, a hazai és az európai állattenyésztés, a nemzetgazdaságban és a humán táplálkozásban elfoglalt jelentős szerepénél fogva, óriási kihívásokkal néz szembe. Annak érdekében, hogy a régió népességének egyre fokozódó állati termék szükségletét képes legyen az ágazat a továbbiakban is kielégíteni új szemlélet- és gondolkodásmódra, a legfejlettebb tenyésztési módszerek kipróbálására és bevezetésére van szükség, az ágazat teljes vertikumában. Az évezred küszöbén elkerülhetetlen, az állattenyésztési ágazat (ipar) globális stratégiájának újrafogalmazása, a biológiai és a társtudományok által kínált korszerű módszerek kipróbálása és elterjesztése, a merészebb, a konvencionális tenyésztési módszerek által kínált lehetőségeket meghaladó gondolkodás.

A vázoltakból eredően, az állattenyésztés előtt álló problémák egy részének a megválaszolása, a különböző lehetséges megoldások megvitatása képezi az első magyar „Ensminger Iskola" egyik célkitűzését.

A konferencia szakmai programjának előkészítése a Szervező Bizottság ülésén elfogadott szempontoknak megfelelően folyt. Széles körű közvéleménykutatás alapján jelölték ki a konferencia előadóit és a tanácskozás témaköreit. A beérkezett javaslatok alapján, a következők kerülnek megvitatásra:

— az állattenyésztés globális feladatai és ezek hatása a magyar és az európai állattenyésztésre,

— a biotechnológiai és molekuláris genetikai módszerek alkalmazásának lehetőségei az állatitermék-előállításban,

— az élelmiszerbiztonság követelményei az állattenyésztéssel és a takarmányozással kapcsolatosan.

A kiválasztott témák és előadók, mindkét fél tetszésével találkoztak. A kiszemelt előadók a felkínált lehetőséget valamennyien elfogadták, megtiszteltetésnek tekintve, hogy a konferencia munkájában közreműködhetnek. A külföldi előadók, egy a konferenciát megelőző szakmai tanulmányúton kapnak betekintési lehetőséget a magyar állattenyésztés mindennapjaiba.

Szükségesnek tartjuk ezen a helyen is megemlíteni, hogy a rendezvény színvonalának megalapozása az Ensminger Alapítvány jelentős anyagi támogatásával (kb. 50 000 USD) és a Gyöngyösi Főiskola hathatós segítségével jöhetett létre. Ennek köszönhető, hogy a világ élvonalába tartozó amerikai, angol, osztrák, német, finn, olasz, japán, orosz és magyar előadókat tudtunk meghívni.

A szervezők tehát joggal remélik, hogy a konferencia, a hazai állattenyésztés kiemelkedő eseménye lesz és tovább növeli az állattenyésztő szakma presztízsét. A konferencián megvitatásra kerülő témakörökből fakadóan, szűkebb szakterületén kívül, az állatorvosi kar, a biológiai társadalom, a táplálkozástudomány, de a humán orvosi kar érdeklődői számára is új ismereteket nyújtó előadásokra kerül sor.

Ezen a helyen is szeretnénk köszönetünket kifejezni azoknak, akik tevőlegesen, illetve jelenlétükkel is hozzájárultak az Ensminger Iskola magyarországi megalapításához. Külön köszönettel tartozunk az előadóknak, hogy jelentős elfoglaltságuk mellett időt szenteltek a konferenciára való felkészülésre és előadásuk megtartására.

A jelen kiadványban közreadott publikációkat sem az Állattenyésztés és Takarmányozás c. folyóirat Szerkesztő Bizottsága, sem a Konferencia Előkészítő Bizottsága nem lektoráltatta. Így azok tartalmáért, mindennemű felelősséget, a szerzők viselnek.

Topel, D. – Marpel, D. – Gere, T.

ORGANISERS OF THE CONFERENCE

TOPEL, DAVID G.

Topel, David G. was born in Wisconsin (USA) and received the BS from the University of Wisconsin, the MS from Kansas State University, and the PhD from Michigan State University. He joined the faculty at Iowa State University where he gained international recognition for his research in pork quality and the aetiology of the Porcine Stress Syndrome. He served as Head of the Animal and Dairy Sciences Department at Auburn University and as Dean of the College of Agriculture and Director of the Iowa Agriculture and Home Economics Experiment Station at Iowa State University. As Dean, he established fruitful exchanges of faculty and students with institutions of higher education in China, Costa Rica, Czech Republic, Honduras, Hungary, Kyrgyzstan, Russia, Slovakia, Ukraine, Uzbekistan and Thailand. Topel is retired but continues to serve as Ensminger Chair for International Programs in Animal Science.

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MARPLE, DENNIS

Marple, Dennis was born in Iowa (USA) and was awarded the BS and MS degrees from Iowa State University, the PhD from Purdue University and did post doctoral studies at the University of Wisconsin, Madison. His research interests include animal growth and meat quality. He is a member, Fellow and past President of the American Society of Animal Science, and has served as Head of the Animal and Dairy Science Department at Auburn University and the Animal Science Department of Iowa State University.

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GERE, TIBOR

Gere, Tibor was born in Hungary, began his education at the Gödöllő Agricultural University, and continued his studies at Moscow Tyimirjazev Academy of Agricultural Sciences in animal breeding. He was Assistant and Associate Professor at the University of Agricultural Sciences, Gödöllö and was awarded his PhD degree in 1970. He was Director of the Research Institute of Animal Breeding, Hungary, and then began working at Gyöngyös where he founded the Animal Breeding and Nutrition Department. He received the doctoral degree from the Hungarian Academy of Sciences, and was elected as a member of three academies of sciences abroad. His research interests include animal genetics, biotechnology, dairy cattle breeding and animal behaviour. He has been an invited Professor in Germany, Egypt and Syria and for two months was a scholar at Iowa State University.

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A KONFERENCIÁT SZERVEZTÉK

TOPEL, DAVID G.

Az USA Wisconsin államában született. Egyetemi tanulmányait a Wisconsin-i és a Kansas-i állami egyetemeken végezte, PhD fokozatát pedig a Michigani Állami Egyetemen szerezte. Szakmai tevékenységét az Iowai Állami Egyetemen kezdete, ahol a sertéshús minőségével és a sertések stresszérzékenységével (Porcine Stress Syndrome) kapcsolatos kutatásai révén nemzetközi elismerést szerzett. Később az Auburn Egyetem Állattenyésztési és Tejgazdasági Tanszékének vezetője, majd az Iowai Egyetem Mezőgazdasági Karának dékánja lett. Ez utóbbi minőségében gyümölcsöző kapcsolatot és diákcsere programot alakított ki számos (Costa Rica-i, honduraszi, thaiföldi, cseh, magyar, szlovák, orosz, kirgiz, ukrán, üzbegisztán) felsőoktatási intézménnyel. D. Topei nyugdíjasként tovább vezeti az Ensminger Alapítvány Nemzetközi állattenyésztési programját.

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MARPLE, DENNIS

Iowa államban (USA) született. Egyetemi tanulmányait az Iowai Állami Egyetemen végezte, tudományos fokozatát (PhD) a Purdue Egyetemen szerezte és post-doktori tanulmányait a madisoni Wisconsin Egyetemen folytatta. Fő kutatási területét a gazdasági állatok húsminősége, a növekedés- és fejlődés vizsgálata képezi. Tagja, és elnöke volt az amerikai állattenyésztők társaságának. Tanszékvezető egyetemi tanárként dolgozott az Auburn Egyetem Állattenyésztési és Tejgazdasági Tanszékén, majd hosszú időn át vezette az Iowai Állami Egyetem Állattenyésztési Tanszékét.

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GERE TIBOR

Gere Tibor egyetemi tanulmányait a Gödöllői Agrártudományi Egyetemen kezdte meg, majd a Moszkvai Tyimirjazev Akadémia Állattenyésztési Karán folytatta. Négy évi főállattenyésztői munkát követően egyetemi tanársegéd, adjunktus, majd docenst a GATE Állattenyésztéstani Tanszékén és 1970-ben szerzett kandidátusi fokozatot. Tíz éven át igazgatóként irányította az Állattenyésztési Kutatóintézetet, majd a GATE Gyöngyösi Főiskolai Karán vezeti az Állattenyésztés- és Takarmányozástani Tanszéket. 1988-ban védte meg akadémiai doktori értekezését. 1997-ben a biotechnológia, etológia, az állatgenetika és a szarvasmarha-tenyésztés területén folytatott évtizedes kiemelkedő kutatómunkájának elismeréséül több külföldi tudományos akadémia (az Orosz Mezőgazdasági Akadémia, a Szlovák Mezőgazdasági Akadémia, a New-Yorki Tudományos Akadémia) tagjává választotta. Fontosabb kutatási és oktatási területei az állatok növekedésének és fejlődésének vizsgálata, a tejelő tehenek takarmányfelvétele és -értékesítése, az optimális testsúly meghatározása, növekedési hormon gén vizsgálata, a tej szomatikus sejtszámát befolyásoló genetikai és környezeti tényezők vizsgálata. Vendégprofesszorként a Német Szövetségi Köztársaságban, Egyiptomban, Szíriában töltött el rövidebb hosszabb időt, valamint két hónapot töltött ösztöndíjas professzorként az Iowai Állami Egyetem Állattenyésztéstani Tanszékén.

Címe: Szent István Egyetem, Gazdálkodási és Mezőgazdasági Főiskolai Kar, Állattenyésztés és Takarmányozástani Tanszék, 3200 Gyöngyös, Mátrai út 36. Tel.: +36-37551-950, Fax: +36-37551-952, E-mail: tgere@mail.gyfk.hu

FITZHUGH, HENRY A.

Fitzhugh, Henry A. is an animal scientist and agricultural systems specialist with experience in agricultural research and development in 45 countries in Africa, Asia, Europe and the Americas. He was the Founding Director General (1995–2001), International Livestock Research Institute, Nairobi, Kenya, which has the global mandate for livestock research to enhance productivity and sustainability of agricultural systems in the developing world. Earlier positions included Director General, International Livestock Centre for Africa, Addis Ababa, Ethiopia; Director, Winrock International Institute for Agricultural Development; and Associate Professor, faculties of animal science, genetics and statistics, Texas A&M University. He has published extensively on research priorities and strategies, agricultural production systems, livestock genetics and management. Fitzhugh received his BS, MS and PhD degrees from Texas A&M University and did post doctorate studies at ARC Animal Breeding Research Organization; Institute of Animal Genetics, University of Edinburgh, Scotland.

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Fitzhugh, Henry A. állattenyésztéssel foglalkozó tudós és mezőgazdasági rendszer-specialista, aki Afrika, Ázsia, Európa és az Amerikai 45 országában szerzett tapasztalatokat a mezőgazdasági kutatás és fejlesztés területén. Alapító vezérigazgatója volt (1995–2001) a Nemzetközi Állattenyésztési Kutató Intézetnek (International Livestock Research Institute), Nairobiban, (Kenya), melynek kiemelkedő szerepe van a haszonállatok tanulmányozásában abból a célból, hogy fokozzák a mezőgazdasági rendszerek produktivitását és fenntarthatóságát a fejlődő országokban. Korábbi beosztásai: az Afrikai Nemzetközi Állattenyésztési Központ (International Livestock Centre for Africa) vezérigazgatója Addis Abebában (Etiópia); a Winrocki Nemzetközi Mezőgazdasági Fejlesztési Intézet (Winrock International Institute for Agricultural Development) igazgatója; egyetemi docens a Texasi A&M Egyetem Állattenyésztési, Genetikai és Statisztikai Karán. Számos tanulmányt készített a kutatási prioritásokról és stratégiákról, a mezőgazdasági termelési rendszerekről, a haszonállat genetikáról és gazdálkodásról. Egyetemi tanulmányait és tudományos fokozatát (PhD) a Texasi A&M Egyetemen szerezte és tanulmányait Skóciában, az Edinburgh-i Egyetemen az Állatgenetikai Intézetben folytatta.

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GLOBAL DIRECTIONS AND SUSTAINABILITY OF ANIMAL PRODUCTION IN DIFFERENT REGIONS OF THE WORLD

FITZHUGH, HENRY A.

INTRODUCTION

Global demand for meat and milk will increase 50 percent by 2020, primarily because consumption of meat and milk in developing countries will double by 2020! The economic, structural, and environmental consequences of this increased demand will be profound, requiring major changes in where and how livestock products are produced, processed and marketed.

The challenge is to ensure that these changes are sustainable-socially, economically and environmentally. Technical and policy research can help meet this challenge by developing the tools, technologies and information needed by producers, processors and political decision makers.

This paper examines the causes and consequences of the projected increase in demand for livestock products — the Livestock Revolution — and

identifies priorities for research and development to help ensure the sustaina-bility of animal production in future.

Livestock production systems

Meat and milk production from the three major categories of livestock pro-duction systems is shown in *Table 1.* Ruminants in pastoral systems, often the only economic means for utilising lands not suitable for cultivation, provide for 8 to 9 percent of global milk and meat consumption. The largest quantities of meat and milk are produced from mixed farming systems; however, pork and poultry meat production from industrial systems is increasing rapidly. Stratified systems involve transfer of livestock between systems for different stages of the production cycle; for example, moving calves from the pastoral cowherds in which they were born to be finished in industrial feedlots (*CAST*, 1999). Produc-tion from stratified systems is included under "Industrial" in *Table 1.*

Table 1.

Percent global production from major livestock production systems

Product	Million tonnes	Pastoral	Mixed Crop-Livestock	Industrial
Meat				
Cattle	53	23	65	12
Buffalo	3	0	100	0
Sheep/goat	10	30	69	1
Swine	71	1	60	39
Poultry	43	2	24	74
Milk	473	8	91	1
Eggs	40	1	31	68

Source: Adapted from *CAST*(1999), based on *Seré and Steinfeld* (1996)

The Livestock Revolution

"Even as population grows at a record pace, those with low incomes, who account for most of humanity and who typically depend on a starch staple, such as rice, for 70 percent or more of their calories, want to diversify their diets by consuming more livestock products. This desire to move up the food chain where more than half the population in developing regions will be urban in 2020 as compared to only 30 percent in 1980 appears to be universal. In every soci-ety where incomes have risen, so has consumption of livestock products." (*Brown, 1995*)

The remarkable increase in cereal production over the last fifty years, known as the "Green Revolution", has rescued millions of poor people in devel-oping countries from the ravages of hunger. But as *Lester Brown* has noted, humans want more than starch staples; there is an almost universal desire for diets including animal source foods. The next food revolution — "The Livestock Revolution" — will stem from this increased demand for meat and milk in devel-oping countries home to four out of five humans (*Delgado et al.*, 1999).

Population in developing countries is expected to increase from 4.5 billion in 1997 to 6.1 billion in 2020; this 1.6 billion increase is greater than the total

population in developed countries (1.3 billion in 1997, 1.4 billion in 2020). Per capita incomes are projected to increase 3 percent per annum in sub-Saharan Africa and 6 percent in Asia and Latin America (*Cranfield et al.*, 1998), and more than half the population in developing regions will be urban in 2020 as compared to only 30 percent in 1980. Demand for nutrient-rich, easy-to-prepare meat and dairy foods will be driven by this increased purchasing power and urbanization in developing countries following the pattern already seen in developed countries during the last century.

Delgado et al. (1999) predicted per capita demand for meat and milk will increase by 50 percent in developing countries, with the largest growth in demand for poultry meat (*Table 2*). These per capita increases, multiplied by population numbers, indicate that total demand for meat and milk will double in developing countries. Only small increases are expected in developed regions, where per capita consumption is already high and population growth will be low. Moreover, the well-publicized allegations of adverse health effects from consumption of animal source foods and concerns about "mad cow" and other livestock diseases continue to discourage meat and milk consumption.

Table 2.

Meat and milk consumption trends for developed and developing regions

Region	Per capita consumption, kg			Total consumption, million tonnes			Percent of global total consumption		
	1983	1997	2020	1983	1997	2020	1983	1997	2020
Developed countries									
Beef	27	23	25	32	30	34	67	52	40
Pork	29	28	29	34	36	39	63	44	33
Poultry	16	22	28	19	28	38	66	49	36
Meat	74	76	84	88	99	114	64	47	35
Milk	195	195	203	233	254	276	66	56	43
Developing countries									
Beef	5	6	9	16	27	52	33	48	60
Pork	6	10	13	20	46	80	37	56	67
Poultry	3	7	11	10	29	67	34	51	64
Meat	14	25	35	50	112	213	36	53	65
Milk	35	44	61	122	198	372	34	44	57

Source: Delgado et al. (2001) with updated statistics from *Delgado et al.* (1999). Meat is uncooked bone-in weight, including mutton and goat as well as beef, pork and poultry. Milk is liquid milk equivalent weight, including milk products but not milk fed to calves. Per capita and total consumption are three-year averages centered on year shown

Updating their earlier analysis, *Delgado et al.* (2001) expect total consumption of livestock products in developing countries to increase 2.8 percent per year in developed countries, compared with only 0.6 percent in developing countries. Meat consumption in developing countries will increase from 112 million tonnes in 1997 to 213 million in 2020, accounting for almost two-thirds of global consumption (*Table 2*). The consumption of dairy products in developing countries will be 372 million tonnes with about one-third imported from developed countries primarily as milk powder and butter.

The key trends, which define the Livestock Revolution, including the demand growth in developing regions and increased market share for poultry, have actually been underway for several decades. Total meat consumption in developing countries increased from only 36 percent of world total in 1983 to 53 percent in 1997 (*Table 2*). Poultry's share of meat consumption has doubled from 15 to 29 percent in developed regions, and from 12 to 26 percent in developing regions since 1967. In North America and Europe, market share for poultry has primarily increased at the expense of beef; whereas in East and Southeast Asia, growth in poultry consumption has been primarily at the expense of pork (*Rosegrant et al.,* 2001). Almost all increases in poultry meat production, in both developed and developing regions, have been from chickens raised in grain-based industrial systems.

Livestock feeds

The amount of concentrates fed, including feed grains and oilseed meals, will nearly double in developing countries by 2020 (*Table 3*). About half the increases in concentrate fed in Asia and North Africa will be imported from North America, Australia, Argentina and Brazil (*Rosegrant et al.,* 2001). In addition, tropical crops which are now primarily subsistence food crops (such as sorghum, sweet potatoes, cassava and other coarse grains, roots and tubers) will be increasingly used for livestock feed.

Table 3.

Cereals fed to livestock in million MT and as % of total cereal consumed in the region

	Cereals fed, million tonnes				% fed of total cereals consumed	
	1983	1993	1997	2020	1997	2020
Developed	465	442	425	493	59	60
Developing	128	194	235	432	21	26
East Asia	52	81	119	233	29	39
Southeast Asia	6	12	15	27	13	16
South Asia	3	4	3	6	2	3
Latin America	40	55	58	98	42	46
WANA	24	29	36	59	28	30
SS Africa	2	3	4	8	5	5
World	592	636	660	925	36	37

Sources: Delgado et al. (2001) and *Rosegrant et al.* (2001)

Cereals include wheat, rice, maize, barley, sorghum, millet, rye and oats. Maize and other coarse grains are the principal grains fed to livestock. Three-year averages are centered on year shown. WANA: West Asia and North Africa; SS Africa: Sub-Saharan Africa

Livestock consume about one-third of the global cereal consumption (*Table 3*). Grain feeding has provoked concerns about livestock competing with the poor for basic food requirements. However, as indicated in *Table 4*, the conversion efficiencies of human edible grain to meat are remarkably high (especially for ruminants in developing countries) because on a life cycle basis most feed consumed by livestock is from human non-edible sources (*Fitzhugh,* 1998b; *CAST,* 1999).

**Life cycle use of human edible grain for meat production by
different livestock species in developed and developing countries**

Species	Grain consumed per meat produced, kg/kg	
	developed	developing
Cattle	2.6	0.3
Sheep/goats	0.8	0.3
Swine	3.7	1.8
Chickens	2.2	1.6

Source: CAST (1999)

Sustainable production

Most meat and dairy products will be produced in the same developing countries where they will be consumed. To a large extent, this is because of limited capacity in most developing countries for cold chain processing, storage and marketing of meat and dairy products. Other factors favouring local production include cooking and taste preferences, lower costs due to lower quality and sanitary standards, religious requirements for slaughter and processing procedures and non-tariff barriers to imports. Therefore, the Livestock Revolution can provide significant income opportunities for smallholders, the majority livestock owners in most developing countries (*Kaufman and Fitzhugh*, 2004).

Part of required increases in meat and milk production will come from expanding numbers, but efficient and sustainable increases will depend on improving per head yields of meat and milk. Significant improvements can be made by extending available information and technologies for reducing losses from reproductive inefficiency, disease and parasites and for increasing productivity through the improved nutrition, management and use of genotypes adapted to local production conditions. However, there is need for research targeted to the constraints specific to livestock production in the predominately tropical developing countries (*ILRI*, 2000).

Production from industrial systems will expand wherever relatively low cost concentrates are available and profitable urban markets justify the greater investment costs for infrastructure, feed and other inputs required by industrial systems. However, the economies of scale attributed to industrial systems are more apparent than real because of tax breaks, subsidies and failure to account for the costs of soil and ground water pollution and greenhouse gas emissions (*Delgado et al.*, 1999). In some developed countries, concern about environmental impacts has lead to legislation to restrict expansion of industrial systems and to internalise the environmental costs through surcharges on feed inputs or meat outputs (*de Haan et al.*, 1997). Severe environmental and public health problems from industrial systems are emerging in developing countries where regulatory controls tend to be weak (*Steinfeld*, 1998). One public health concern is that industrial systems, concentrating large numbers under often-unsanitary conditions, are incubators for disease, including zoonoses such as influenza. Moreover, the use of antibiotics as feed additives and to control diseases of intensification has been linked with development of antibiotic resistant disease strains (*Perry et al.*, 2002).

Priorities for research and development

Priorities vary across species, systems, agro-ecologies and geo-economic factors, and they also differ depending on whether R&D will be funded by the private or public sector. For the mature markets of developed regions, priorities include improving food quality and safety, controlling zoonotic disease and mitigating environmental degradation; whereas, for developing regions, the urgent priority is increasing productivity to meet demands of the Livestock Revolution. The challenge will be to achieve these increases in ways that also reduce poverty and protect the environment in developing countries (*ILRI*, 2000).

The traditional animal sciences have an important role to play, including the transfer and adaptation of results from the generally better-funded research in human nutrition, health and genetics. The needs of future generations must also be addressed through research to conserve biodiversity and improve management of natural resources. To an increasing extent, future advances in productivity and sustainability will depend on contributions from the agronomic, environmental and socioeconomic sciences, generally working with animal scientists in interdisciplinary teams.

Biotechnology, building on research in genomics and proteomics, will be increasingly important despite concerns about GM foods that are primarily voiced by the well fed in wealthy countries, not by the poor and hungry in developing countries. However, genetic modification of animals will more likely be used by the pharmaceutical industry to develop products for the human health market with the greater impact of biotechnology on animal production through development of diagnostics and vaccines, microbial control of pollutants from industrial systems, and improved macro- and micro-nutritional value of forages, feed crops and food crop residues.

Regulations and policies increasingly impact on the success and sustainability of livestock enterprises; for example, the decisions to restrict beef exports from Canada (and earlier from the U.K.) when cattle afflicted by BSE were identified. Less dramatic, but with severe consequences for livestock producers in developing countries, have been policy decisions in some developed countries to subsidize their producers while dumping surplus meat and dairy products in developing countries. Too often, regulatory and other policy decisions serve the near-term special interests of a select few with little consideration to the long-term costs and consequences for the majority of producers and consumers. For example, to reduce poverty and hunger in developing countries, there is particular need for policies, which improve access by smallholders to credit, input-output markets and livestock services.

Priorities for bio-technical and socio-economic research and development to improve productivity and sustainability of animal production are addressed in more detail by *de Haan et al* (1997), *Fitzhugh* (1998a), *CAST* (1999) and *ILRI* (2000).

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SUMMARY

Most humans prefer diets that include animal source foods. Four out of five humans live in developing countries where per capita consumption of milk and meat is 20 to 30 percent of that in developed countries. However, overall demand for meat and milk in developing countries will double by 2020, with only small increases in developed countries. By 2020, developing countries will account for 65 and 57 percent of global meat and milk consumption. This demand growth — the Livestock Revolution — will be driven by improved purchasing power, urbanization and population increases. The amount of grain fed in developing countries will also double, primarily for feeding swine and poultry in industrial systems. Forages, crop residues and other human non-edible feeds will remain the primary nutrient sources for ruminants in pastoral and mixed farming systems. The Livestock Revolution will bring new market opportunities to producers in developing countries, including poor smallholders; however, it will also pose significant public health and environmental problems in countries where regulatory controls are weak. Targeted technical and policy research is needed to help ensure that the opportunities and challenges from the Livestock Revolution are successfully met.

GLOBÁLIS TRENDEK ÉS FENNTARTHATÓ FEJLŐDÉSI STRATÉGIÁK A FÖLD ÁLLATTENYÉSZTÉSÉNEK KÜLÖNBÖZŐ RÉGIÓIBAN

FITZHUGH, HENRY A.

ÖSSZEFOGLALÁS

A legtöbb ember az állati eredetű táplálékot részesíti előnyben. Ötből négyen fejlődő országokban élnek, ahol a fejenkénti tej- és húsfogyasztás 20–30%-a a fejlett országokénak. Mind ezért, a hús és tej iránti kereslet a fejlődő országokban meg fog kétszereződni, míg a fejlett országokban ez a növekedés csekély lesz. 2020-ra, a fejlődő országok hús és tej fogyasztása, a globális fogyasztás 65 és 57%-át fogja kitenni. Ezt a keresletnövekedést — az„állatitermék-előállítás forradalma" — a megnövekedett vásárlóerő, a városiasodás és a népességszaporulat fogja előidézni. A cereáliák felhasználása is meg fog kétszereződni a fejlődő országokban, elsősorban a sertés- és baromfi ipari méretű termelése következtében.

Egyes takarmányok, valamint melléktermékek és más, emberi fogyasztásra alkalmatlan , elsősorban zöld táplálékok megmaradnak a kérődzők és vegyes gazdálkodói rendszerek számára, ahol elsődleges tápanyagforrás a legeltetés. Az „állatitermék-előállításának forradalma" új piaci lehetőségeket teremt a termelőknek a fejlődő országokban, beleértve a szegényebb magán kistermelőket is. Ez a nagyarányú fejlődés azonban jelentős egészségügyi és környezetvédelmi problémákat vet fel azokban az országokban, ahol a szabályozók nincsenek kidolgozva és a felügyeletek gyengék. Célirányos stratégiai kutatások szükségesek ahhoz, hogy az állattenyésztési forradalom kínálta lehetőségek és kihívások sikeresen találkozzanak.

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ETHICS AND COMMUNITY OF LIFE IN ANIMAL PRODUCTION

HODGES, JOHN

INTRODUCTION

Ethics is a new topic in agriculture. Indeed, it is a new topic in western society generally. Consider the following events: in 2002, the European Parliament held "The First European Ethics Summit"; in 2003, the European Union (EU) set up a "Reflection Group on Spiritual and Cultural Dimensions of Europe"; shortly after taking office in 1996 the President of the World Bank, James Wolfensohn, convened an international conference in Washington DC on "Moral and Spiritual Values in Development"; in 1993 the United Nations established the first Commissioner for Human Rights; the European Society for Agriculture and Food Ethics (EurSafe) was established in 2001 as an independent professional society to provide a focus for ethical issues in agriculture and food; many national Animal Science Societies have recently set up professional Ethics Groups and now invite papers at their conferences and in their journals, for example: U.K., USA, EAAP and WAAP.

Why have Ethics moved suddenly into prominence in public thinking, writing and debating? And what is the relevance to agriculture and food? A general reason is the notable increase in misbehaviour and abuse of knowledge, resources and finances by leaders in some public institutions. The increasing number of these scandals raises suspicions in the mind of citizens that some leaders in public institutions cannot be trusted to make good decisions in the interests of society as a whole. Personal and self-seeking financial agendas have been revealed which cause loss, suffering and exploitation to many individuals and to some developing countries. Even some watchdogs of financial propriety, public auditors, have been found in collusion with senior executives in

public companies to deceive and to defraud investors, shareholders, consumers and the public generally. Trust has been eroded. Ethical behaviour is no longer simply a private matter. Today ethical behaviour is in the public arena.

In the context of agriculture and food there is a further component in this matrix of growing concern about ethics. People are aware that emerging bio-technologies are being implemented by sectional interests often without public knowledge, debate or informed consent. Intelligent people well understand that new ways of trying to solve intractable problems are needed to enable society to advance. Molecular biology promises to open some of these doors. However molecular biology involves manipulating fundamental life processes and thereby is capable of reshaping traditional food security, safety and quality. Fear and suspicion have arisen because the decisions to use new biotechnologies in the food chain are made largely by multi-national companies often with the partisan support of governments. While the stated aim is always improvement in the quantity, quality or cost of food, everyone knows that the key decision-makers are motivated by their mandate to maximize share-holder benefits. This situation raises ethical questions about biotechnology in the food chain. Food is not an optional extra for life; further people in the West can no longer grow their own food.

What are ethics?

Socrates provided a useful definition: "Ethics is knowing the difference between good and evil and then choosing to do the good". The earliest philosophers and religious teachers all recognized that human civilization is a community experience. In pondering the impact of individual and institutional moral behaviour they conclude almost without exception that any human society is doomed if its members do not practise a positive ethical code. Races and nations may successfully make war on other communities but, without ethics, survival of their own community is at risk.

Religion and tradition have set the norms of acceptable behaviour in most societies. Some have proposed that religion was invented by society to provide a moral structure in human society. However, the three monotheistic faiths state that acceptable moral behaviour for individuals and for society is revealed by God.

Civilized nations generally codify some of their religious and traditional ethical standards into laws with sanctions within an ethical judicial system as safeguards against abuse.

Origin of ethical standards in the West

In Europe, and by extension in most of the Western world, most private and public ethical standards have derived from Judea-Christian teaching. The historical process has been irregular and punctuated by some terrible misbehaviour in the name of Christianity. But despite these aberrations, over the last 2000 years the cultures of European and hence of American countries were slowly colonized by Christian values. In the 19th and 20th centuries these countries became recognized not only for economic success but also for ethical be-

haviour, justice and internal peace. Despite some brutality against other nations, they held positive ethical standards and assumptions on how decent people should behave.

Moral behaviour in the European and descendent nation-states has thus been based upon Biblical principles, practiced by accepted custom and under the rule of law. In earlier centuries limited economic freedom and a strong social hierarchy were also bulwarks against anti-social behaviour. Of course, the brokenness of human nature means that the ethical code was not always practised; but basic ethics in society as a whole was summarized by Jesus in the Golden Rule: "Treat others as you wish them to treat you". When there were no laws on some topics the Golden Rule was an unwritten law of normal behaviour. When "Good Ethical Behaviour" was an assumption in society, there was little need to speak about the obvious. Thus ethics was not a common topic of conversation or debate. Good and evil were identified by the Bible and, where needed, were turned into laws, which defined right, and wrong. Western society was ethically stable.

Major shifts in Western morality

This stable ethical situation in Europe and its cultural colonies has been shaken by major shifts in worldview over the last few centuries. The twin bastions of Christian religion and of traditional ways of behaving have been challenged.

First, the Enlightenment brought many new perspectives about the nature of life and in so doing, challenged the religious foundations of society. It was a slow process with many compounding events including the birth of modern science. But over several centuries the Enlightenment process shook the stable religious foundations of society and gradually removed God from public decision-making.

Second, during the 20th century, traditional ethical assumptions and practices have faced a variety of new challenges including liberation movements, existentialism, post-modernism, post-Christian values and the allure of market economy *capitalism which*, at its roots, encourages short-term self interest. Behaviour that had been regarded as normal was no longer obvious to everyone. The shift towards a materialistic view of life has contributed to the break-up of more traditional, structured society. New assumptions on what is normal for education, travel, family life, wealth and employment have emerged. Belief in God and religious faith have been moved to the private areas of life. Although 70-80% of people in the West say they believe in God, the goals of material prosperity dominate most decision-making. Further, some intellectuals, significantly lawyers, consider that morality has no absolute foundations and is simply a relative concept. Relative morality offers the option of anti-social behaviour without sanctions. As G.K. Chesterton, a British writer said, "When people stop believing in God they do not believe in nothing, they believe in anything".

Western ethics at entry to the 21st century

Where are we today? As the 21st century dawns, post-Christian Western society is dismantling its own traditional moral foundations. This is bad news in the long-term. The 20th century provides many examples of bad behaviour exploding when the constraints of just law, ethical leaders and the Golden Rule are removed. In summary, ethics is in a bad way.

Recently I asked the President of the Ukrainian Academy of Medical Sciences in Kiev how ethics are decided in post-Soviet society. "Listen" he said, "The strictly enforced ethic of communism which subjected everything to the Party has gone. Today, in Ukraine, everyone makes up their own ethics". Today in the West too, we have arrived at that same smorgasbord of ethics, though from a different historical process.

Ethics and biotechnology

"Do-Your-Own-Ethics" has serious implications for the use of biotechnology. The interests of people worldwide and in future generations must be paramount when molecular biology is considered for use on a large-scale in the food chain. Decisions based upon sectional interests without in-depth experience of impacts upon all other parties are, by definition, self-serving. Failure to identify with the interests of others can have tragic, long-term and irreversible consequences. The following list shows the disparate variety of proposals on who should decide the use of biotechnology in food: Use public opinion surveys; leave decisions to scientists; leave decisions to multi-national companies; use every available technique and let mankind become master of its own future evolution and fate; let the market decide; set up groups of wise men and women; invite the public into consultation processes; ask the World Trade Organization; leave decisions to representative governments; pass UN resolutions; ask religious leaders.

The move from accepted standards of ethical behaviour rooted in Judea-Christian moral teaching to floating ethics and relative morals is a serious change in the fabric of human community. Thinking people recognize this and consequently courses on ethics are being introduced in professional training. In the last ten years all business schools have introduced Ethics, often as a required course. Governments, the EU, the President of the USA, professional bodies, big-businesses and almost any public body now have their ethical advisors. McDonalds, swiftly followed by Burger King, have recently established ethical advisory panels to guide them on animal welfare.

Ethics, biotechnology and the food chain

The foregoing brief summary of ethical origins and changing values in recent centuries in the West provides a background against which we may consider the position of agricultural scientists today in relation to biotechnology in the food chain. The issue chosen as an example is the impact of biotechnology upon the welfare of livestock. In the decades ahead what ethical basis will be used to determine the use of innovative molecular biology techniques for manipulating livestock?

Animal welfare movement

As with many ethical issues, the original call for more humane treatment of farm animals started with individuals and NGOs about 40 years ago. The protests focus against lack of adequate care for animals in what is called "factory farming". It is an emotive issue as people easily identify with large mammals. Vegetarians and vegans often give abuse of animals as the reason for their diet. Unfortunately there are some violent extremists. Nevertheless, thoughtful people recognize the way we treat food animals is a valid ethical issue. Some key early publications are: *Godlovitch et al.* (1972) and *Singer* (1975).

Why has traditional livestock farming changed so markedly? It is because the values now driving livestock intensification derive from market economy capitalism and include: improved productivity, increased economic efficiency, higher returns on capital investment, reduced labour costs, greater throughput per unit time and space. Today these economic values influence the research agendas of animal scientists who target goals such as: higher prolificacy, lower mortality, increased genetic gain in production traits, lower nutrition costs, better conversion rate, increased biological efficiency, improved ratio of edible to waste tissue, less variation between animals, shorter time from conception to market etc.

Since the end of the Second World War, these market values and associated scientific objectives have driven livestock into large-scale, automated. Maximum throughput per unit time and space leads to higher profits and returns on investment. This is not a small business. In 2002, ten billion mammals and birds were produced for food in the USA. For more recent documentation of factory farming see: *Eisnitz* (1997) and *Scully* (2002).

Protests about poor comfort levels and quality of life from the animal welfare/rights/liberation movements have brought about a variety of legislative responses with minimum standards for housing, space, building design features, transport and slaughter processes. The legislative action has been greater in Europe than in the USA (*Singer*, 2003).

Ethics of animal welfare

Human values attached to domestic mammalian and bird species have changed from traditional care within the community of life to ethics of utility and convenience. Proponents of animal welfare argue that livestock production now operates unethically as it fails to take account of the experience of animals as sentient beings. They point out that, although animals can respond to direct pain, they are unable to express their needs for normal body movements and lifestyles. The counter-position considers that intensive systems do not inflict physical pain; that basic needs of food, shelter and disease control are provided; and further, that animals do not need to experience quality of life as humans define it. Detailed angles in this debate are not elaborated here but recent publications include: *Scruton* (2002); *Cavalieri* (2002); *DeGrazia* (2002).

Species and animal welfare

There is however one over-riding principle at the heart of this debate. It is far deeper than how many cubic centimetres a chicken needs. *The major issue is the species argument.* This is not simply a philosophical issue. It is the hinge, which will determine the ethical treatment of livestock animals in the emerging era of molecular biology.

Species boundaries are defined by reproduction, which takes place within and not between species. Thus, typically the genome of the species, although carrying genetic variation in the form of alleles, is a confined gene pool. Cross-fertilization between species is not normal. That is the way things are.

People wanting more care for farm animals recognize species boundaries and argue that mammalian livestock species, for example cattle, have their own intrinsic rights in the same way as members of the human species. In Europe this position resonates with historic Christian culture, which calls man to account for the care of livestock species used for food (Bibie: Genesis, 1). The arrival of human, ethnic and womens' rights movements in recent decades has encouraged the concept of rights for subsets of humanity and therefore fosters the concept of rights for the animal species with whom humans closely identify in the community of life.

The species boundary is equally important, for a different reason, to people in the driving seat of further intensification. They consider the human species to be superior to livestock species and believe the former may use their power over the latter, even by keeping animals in unnatural conditions. Although rarely articulated in words, this position is declared by actions in the ongoing promotion of intensification and scale. This view is not based solely upon livestock being "dumb"; it is made clear by the care humans give to disadvantaged individuals whose abilities are even less than normal livestock. This behavioural ethic, based upon the species boundary, springs from a deep belief that humans are of more value than livestock.

Species and molecular biology

Molecular biology undoubtedly challenges the way people think about themselves as humans and about animals. The Human Genome Project now offers identification of all the base sequences in the human genome; and comparisons with some mammals show a very high level (>95%) of correspondence. What then is the meaning of being human? If the molecular boundaries between Homo sapiens and domestic livestock are so minimal are they really of any consequence? Is it any longer valid to behave as though dumb and incapacitated humans are worth more and should be given unlimited care whereas farm animals may be treated ignominiously?

The break-through in molecular biology has placed unprecedented power in the hands of the human species. The current batch of biotechnology techniques is only a small sample of the endless vista of options which now lies within the grasp of humanity for reshaping the genetics of every living thing. Somatic cloning, transgenic organisms, embryo manipulation, somatic stem cells, deletion and insertion of genes — these are but the beginning. The central

dogma of molecular biology is that DNA, together with proteins, controls the genetic characteristics of every living organism. The new tool of biotechnology is the ability to identify functions and traits linked with specific DNA and proteins and then move them across species boundaries. This prospect is a challenge to all ethical codes built upon species boundaries whether currently for or against intensification. It also demands a more perceptive definition of mankind and our responsibilities for the community of life.

Where are we taking our animals?

It is not difficult, but horrifying, to visualize a future scenario of intensive livestock production extended without ethical limits. Projecting such a scenario using current scientific knowledge is likely to be faulty in detail because molecular biology is accelerating so rapidly. But, science, business and society being what they are, we know that scientists will continue to research and to develop more powerful techniques for molecular manipulation. *The real issue is whether their use upon livestock will be ethical and what will be the basis of those ethics.* To enable us to face this issue here is one possible scenario of the future.

Uneconomic parts of the anatomy, such as legs, have been deleted reducing the amount of space needed per animal and also making better use of nutrient input. Genes for meat tissues with higher commercial value, such as beef, are inserted in pigs which, having a more efficient digestive systems than ruminants now produce beef muscle. Genes for feathers and wool have been deleted from food animals and animal skin has been thinned to minimal levels needed to contain functioning tissues. Genes for beaks and some parts of the digestive system have been deleted as nutrients are fed into the body as a drip. Small birds like quail with low body maintenance and without legs have genes from domestic hens and produce large eggs. All animals are linked permanently to a flow-line of concentrated feed laced with appropriate hormones and enzymes. Growth hormone genes have been inserted to speed growth of meat animals. Blood supply to each batch of animals is produced by special animals devoted to that purpose. A blood circuit flows around each batch of cloned animals with links to each animal thus providing control of all physiological functions and bringing the batch to market with perfect economic timing. Some animals, now designed to consist mainly of a rumen, are adapted to processing animal waste which is itself far less than in natural conditions, and which is then recycled in the production line.

With the ability of move genes from any species of animal, fish, insect, plant or microbe genes which improve efficiency are moved into food producing animals. Maintaining body temperature is expensive. So, intensive livestock either have reduced body temperature or in some cases have been turned into cold-blooded creatures. A major breakthrough has at last been achieved in making the slow and uncertain process of mammalian reproduction more efficient. Cloned embryos carrying the most highly productive genes for desired traits are produced in bulk using duplicate genes from either a male or a female parent to produce offspring of only one sex according to the product desired. These embryos are stored in deep freeze ready for a shift in market demand. Embryos are now grown for their early life in an artificial uterus.

A basic animal framework with required genes for the life functions has been developed and can be used as a template for producing a variety of animal products. This basic animal framework needs a brain for neural control of tissues but the head no longer needs mouth, eyes, or ears. A production line of these animal frameworks is maintained and somatic stem cells are added to produce the meat tissue currently required. After harvesting more stem cells are added to regenerate a further crop of animal products. The animal frame is also maintained over long periods using somatic stem cells to replace worn out parts.

Is this all a fantasy? Such scenarios are an extension of scientific and economic reductionism without ethical limit. This value system fails to understand the integration and beauty of nature and the community of life as it now exists based upon DNA. It may be difficult today to grasp the immense power of molecular biology for merging animal species. But these goals and many more are natural extensions of today's research agenda and capture the mindset and excitement of research scientists. This research is very well funded by public and private sectors — perhaps only ever exceeded by nuclear physics and space programmes — in which governments control both the funds and the ethics of use. Who will decide the ethics of animal production?

Where are our ethics taking us?

The future use of emerging biotechnology with livestock has enormous consequences for humankind with huge irreversible consequences, which will either advance human civilization or bring about the downfall of advanced human society. The decisions made will reveal not only how we view animals but also how mankind sees itself. As the ancient philosophers observed, ethics determines the nature of human communities and therefore their chances of survival. In our era if we blindly follow the present utilitarian ethic we may survive but the question is whether we shall still be human.

The crucial issue, to my mind, is to recognize that the boundary separating mankind from other species gives us unique responsibilities as well as privileges. The accumulated wisdom of mankind until the last few generations in the West has always recognized transcendence as part of human nature. The majority of people still do. This transcendence gives us the moral capacity to use our superior knowledge for good or for evil. Our moral framework and ethical decisions will determine our survival as transcendent responsible beings or simply as another animal species.

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SUMMARY

In the last decade, ethics has emerged as a new crucial aspect of agriculture. The reasons for this include the recent bad behaviour by some leaders in public institutions and also consumer fears that biotechnology is being introduced to the food chain by sectional interests without informed consent. The origin of normal standards of ethical behaviour in the culture of Western society is traced to the influence of Christianity. The Golden Rule given by Jesus "Treat others as you wish them to treat you" became the basic norm both for law and for assumed expectations of behaviour in private and in public life. However, the underlying religious and traditional structures for this basic ethical behaviour have been eroded first by the Enlightenment and then, in the 20th century, by new challenges to cultural traditional practices. Consequently Western traditional ethics are drifting, smorgasbord ethics are increasing and community of life is no longer seen as an essential basis for civilization to survive.

The introduction of molecular biology to agriculture requires an éthical basis and it is not clear where this will be found. Animal welfare is taken as a case study to illustrate the problems. Animal production is now being driven by market forces resulting in more intensification and scale. The basis of the animal welfare protest movement is evaluated together with the views of those leading intensification. It is shown that species boundaries are critical to the arguments on each side. A future scenario of animal production is visualized if the present trend of reductions science and economics are continued. In the absence of an alternative to the current values of utility and convenience, it is concluded that man's treatment of animals will raise deep questions about the nature of mankind and the future of civilization.

AZ ÁLLATI TERMÉK-ELŐÁLLÍTÁS ETIKAI ÉS ÁLLAT JÓLÉTI ASPEKTUSAI

HODGES, JOHN

ÖSSZEFOGLALÁS

Az elmúlt évtizedben az etikai szempontok a mezőgazdaság kulcsfontosságú aspektusaiává váltak. Ez többek között a közintézmények vezetőinek etikátlan viselkedésének köszönhető, valamint a fogyasztók azon félelmeinek, hogy a biotechnológiát, különböző érdekeltségek miatt vezetik be a humán tápláléklánccba. A nyugati társadalmak erkölcsrendszere a kereszténység tanításain alapul. Jézus tanítása „Úgy bánj mással, mint ahogy veled szeretnéd, hogy bánjanak", mind a jogrendszer, mind a privát és társas élet elvárt viselkedési normájává vált. Az erkölcsös viselkedés vallásos és tradicionális alapjait azonban, először a Felvilágosodás, majd a 20. századi új kihívásai kezdték ki. Ennek következtében a hagyományos nyugati etika sodródik, és a közösségi életre, már nem mint a civilizáció fennmaradásának legfontosabb alapelveként gondolnak.

A molekuláris biológia bevezetése mezőgazdaságba új erkölcsi alapot kíván. Az előadó, az állatok jóléte témakörét választotta, hogy mintegy esettanulmányként illusztrálja a keletkező problémákat. Az állattenyésztési ágazatot ma elsősorban a piaci tényezők befolyásolják, ennek ered-

ménye a nagyobb intenzitás és a növekedés. Az előadó az állatvédő mozgalmakat is értékeli, összevetve azoknak a nézeteivel, akik az ipar termelését szorgalmazzák. A faji korlátok kérdésköre mindkét fél érveiben kulcsfontosságú. Szó esik még az állattenyésztés jövőjéről, feltételezve, hogy a jelenlegi tudományos és gazdasági trendek folytatódnak. Ha nem találunk alternatívát a haszonelvűség és kényelem jelenlegi értékrendjével szemben, a végkövetkeztetés szerint, az állattartás módszerei komoly kérdéseket vetnek majd fel az emberiség természetével és a civilizáció jövőjével kapcsolatban.

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PROTECTION OF GENE RESOURCES AND THE FREE EXCHANGE OF INFORMATION: THE ROLE OF PATENTING IN ANIMAL BREEDING AND GENETICS

ROTHSCHILD, MAX F.

INTRODUCTION

While herdsmen have practiced the art of animal breeding since the beginning of domestication of livestock, the science of animal breeding is just now entering its second century. From its roots in Mendelian inheritance to the developments in quantitative genetics our field has grown to include modern molecular sciences. Animal breeding as commerce has always "protected" its endeavours commercially by developing breed societies and using pedigrees to protect the intellectual property developed by the master breeders or by forming companies and holding germplasm. The advent of molecular biology, sequencing of genomes and the development of cloning, have brought with them vast sums of money that have been invested to genetically improve animals. With these investments have come the expectations, by companies, of increased rewards and the reality that technical improvements must be protected. However, developing countries fear the loss of local breeds and the possibility of limited access to data. This paper discusses forms of intellectual property, including patents and patent law, and to suggest ways it might impact the field of animal breeding and genetics.

Forms of intellectual property

Intellectual property (IP) is the personal property resulting from the creative or "inventive" work of an individual or individuals. The protection of IP is described in a large body of law that includes copyrights, trademarks, trade secrets, and patents. Copyrights protect IP that includes articles, books, web pages, computer software and music. Creative work can be implicitly copyrighted by merely adding the © symbol, but formai legal protection is made by registering the mark. Trademarks are protection used to identify the source of the owners' goods, products or services. A good example of a trademark in animal breeding is the HAL-1843™ that protects the gene test for porcine stress syndrome. A trade secret represents IP that is not divulged by the owner and therefore confers some competitive advantage. Trade secrets have no time constraints, as long as the information is kept secret. An example of a trade secret might be specific information to make specialized synthetic lines in poultry. Trade secret law (called the Uniform Trade Secret Acts in the US) can serve to protect a technology from theft but the degree of protection is dependent on the technology and the means used to keep the information secret.

Patents represent the largest form of IP and their development is rooted in a large body of law both in the US and abroad. Such laws and their interpretation are subject to change. The descriptions that follow are meant to be general in nature. Patents were developed to "promote the progress of science and useful arts, by securing for limited times, for authors and inventors, the exclusive rights to their respective writings and discoveries" (US Constitution, Article 1, Section 8). Patents are meant to reward the inventor for a new and useful invention. A patent, once granted, represents a legal monopoly granted by the respective country's govemment to an inventor (specific to a geographic region), that permits the inventor to prohibit anyone else from making, using, or selling his or her invention for a specific period of time. The government grants the patent owner a temporary right to exclude all others from making, using, or selling the invention during the term of the patent (now 20 years). Infringement of a patent is a civil wrong and the offended inventor may sue or seek other legal remedies to stop infringement. Patents are intended to protect but not withhold technical information. Patents are published in order to expand scientific development. Patents are very different than published papers or other such works. Inventors are not like co-authors in that they must take part in the inventive process. Patents are not peer-reviewed but are instead assessed by patent examiners as to whether the application meets the requirements of patent ability. A patent must reveal sufficient details, called enabling information, which allows "one of reasonable skill" to duplicate the invention. Other criteria include no obviousness or the inventive step which requires that there be a real invention and not a simple result from some obvious extension of existing technology. Novelty is the second requirement and refers to something created that is new. The third requirement is utility or usefulness of the invention. The breadth of a patent is developed in the claims section and as such, some patents, like gene marker patents, may enjoy very wide coverage across species while others may be limited to single polymorphisms in one breed. Patents may

be related to a process, a product produced by a process or dependent on another patent. Patents are open to interpretation by both examiners and lawyers and the process is a long one. In 2003, there are will be over 350,000 applications and the backlog is about one half million applications. In the US, patents can be applied for up to one year after publication or public disclosure but this is not allowed in most other countries. Patents must be promoted and protected and can be viewed as tradable assets and licensed or assigned to third parties.

Confusion often exists relative to "international" patents, which actually do *not* exist (see http://www.cambiaip.org). Under the Paris Convention Treaty, a group of countries agreed to work with each other to provide a unified examination process and to make filing in its member countries easier by forming the World Intellectual Property Office (WIPO). In unison they adopted the one-year period in which to file an application in one of the other countries without losing the benefit of the original filing date. Using this procedure avoids the citation against the applicant of any "art" that became known after the original fiiing date in the country of origin, but before the fiiing date in another country. Under this Patent Cooperation Treaty (PCT), an applicant is allowed one year to file at the WIPO office and, by designating member countries, holds the legal rights and original filing date in those designated countries without having to file in each country or pay the national expense up front. Although there are fees associated with the PCT application, they are less than one would pay for the foreign national filings at a later date. To obtain a patent in these countries, the application does eventually need to be filed in the national patent offices, fees paid, translations done and the laws and regulations of each country office followed. The applicant may have additional time from the national filing date to request examination, depending on particular country requirements. Delaying examination may provide the time desired to continue development and/or commercialisation; however, during this period, fees called "annuities" must generally be paid. Due to the expense involved, applications are generally filed only in countries where protection is really needed.

A brief history of patents

The early protection of intellectual property rights can be traced back to Venice in the Middle Ages when master craftsmen prohibited competition from former apprentices for a period of 20 years. Such laws had considerably different economic effects for the master craftsman, the apprentice and the general public. In terms of protection of IP associated with animals, early breed societies were in the most general form designed to protect the intellectual property of the master breeders. The first modern patent act is often thought of as the US Patent Act of 1790. There was similar legislation in France in 1791. Patents related to living matter are relatively new. One of the earliest patents for living matter was granted to L. Pasteur for a yeast strain but this was done under the belief that it was an inanimate object and not living (*Lesser*, 2002). The first specialized patent law applied to living organisms was that of the Plant Protection Act of 1930 in the US and provided what is commonly referred to as Plant Breeders Rights (PBR) to propagate new varieties by asexual methods. In 1961, a similar law was passed in France called the UPOV (International Union

for the Protection of New Varieties of Plants — Union Internationale pour la Protection des Obtentions Végétales; http://www.upov.int). Protection in the US was expanded in 1970 with the Plant Variety Protection Act to include sexually reproduced plants. The UPOV was revised in Europe in 1991. Under these laws two principles, "breeder's rights" which allows breeders to use protected varieties without permission of the owner and "farmer's privilege" which allows farmers to collect seeds from their crops and use them, were developed (*Lesser,* 2002). For years many seed companies have attempted to halt this latter practice by asking farmers to sign contracts prohibiting it. Recent molecular genetics technology employed by Monsanto, like the "terminator" technology, biologically prohibits the practice.

The molecular age presented real problems for the protection of IP related to animals. In 1975, a French company failed to patent a "dwarf, egg-laying chicken hen produced by a process" involving a sex-linked recessive gene. In 1980, the United States Supreme Court in a 5–4 decision (*Diamond v. Chakrabarty*) declared that "anything under the sun that is made by man" is patentable. This case concerned the patenting of genetically engineered bacteria that eat oil sludge. In 1987, the US Patent Trade Office issued a pronouncement of the patentability, in principle, of non-human multicellular organisms that were not naturally occurring. This was quickly followed in 1988 by the landmark patent on the so-called "Harvard mouse" which was engineered to be susceptible to cancer. In Europe, similar laws were passed allowing patenting of animals and animal-related inventions, but in some countries like Canada the process is patentable but not the animals. From 1995 to 2001, a total of 45 animal patents were granted in the US (*Lesser,* 2002).

Animal breeding and genetics — IP protection

The broad area of biotechnology encompasses many of the patent applications in our field and the US Supreme Court has established guidelines that apply to this technology (*Nebel et al.,* 2002). The Court made it clear in *Brenner v. Manson* that patent utility implied usefulness and not just "any invention not positively harmful to society". The Court expressed reservations regarding a monopoly on compounds with unknown functions, and that utility must extend beyond proving that the product is a product of scientific research (*Nebel et al.,* 2002).

A list of items in the field of animal breeding that may require IP protection include: genetic markers for genetic improvement, statistical methods for genetic improvement, transgenic and cloned animals, expressed sequence tags, ultrasound methods to measure traits, electronic methods to identify animals, computer software and other written materials. Manuscripts, web pages and software can be copyrighted. Other forms can be protected using trade secrets or by patenting. Some companies have employed the trade secret approach, while others have used patents to protect their research and to be used as marketing tools. Searching for published patents can be made at the U.S Patent and Trademark Office (http://patents.uspto.gov/) and the European Patent Office (http://ep.espacenet.com). At one time US applications were not published.

However, they are now published both in the US and in Europe 18 months after the application has been filed. A sample list is in *Table 1*.

Two noteworthy patents, US patents 4,683,195 covering PCR and 5,582,979 covering dinucleotide repeats, have extremely broad coverage and affect gene discovery and use of many types of genetic (microsatellite) markers. Perhaps the best-known and largest single royalty-generating patent in animal breeding was patent 5,358,649 involving HAL 1843™.

Table 1.

Examples of patents[a] involving common methods, genes and genetic markers in livestock

Species	Date	Patent no.	Title
Chicken	1998	US 5,707,809	Avian sex identification probes
Cattle	1991	US 5,041,371	Genetic marker for superior milk products in dairy cattle
Cattle	1997	US 5,614,364	Genetic marker for improved milk production traits in cattle
Cattle	2001	US 6,242,191	Methods for assessing the beef characteristics of live cattle
Cattle	2001	US 6,284,466	Double muscling in mammals
Sheep	2001	US 6,306,591	Screening for the molecular defect causing spider lamb syndrome
Pig	1994	US 5,374,526	Method for determining genetic marker for increased pig litter size
Pig	1994	US 5,358,649	Diagnosis for porcine malignant hyperthermia
Pig	2001	US 6,183,955	Methods for determining the coat colour genotype of a pig
Pig	2001	US 6,291,174	DNA markers for pig litter size
All	1987	US 4,683,195	Process for amplifying, detecting, and/or-cloning nucleic acid sequences
All	1996	US 5,582,979	Length polymorphisms in $(dC-dA)_n.(dG-dT)_n$ sequences and method of using the same

[a] This represents a sample of patents and applications

There has been some debate in the scientific community as to the validity of the HAL patent since the result seemed obvious once the gene became a candidate. Indeed, the HAL invention was predicted in publications where the strategy for finding the mutation was developed. However, this opinion was based, at least in part, on a misunderstanding of the term obviousness as required for patentability. Patent 5,374,526, which was a method to use ESR gene polymorphisms to improve litter size (*Rothschild et al.*, 1994) stirred considerable debate, not only on the scientific merit of the method, as it was the first to claim use of a marker for a quantitative trait, but also because the patent had been exclusively licensed to one breeding company.

Some confusion existed early in the development of patents in animal breeding as to whether the genes were patented or whether a process or method involving genes and markers was being patented. This was particularly evident in the discussions that followed the ESR patent application (*Rothschild and Plastow*, 2002). However, the issue of patenting gene sequences has raised both legal and commercial concerns. This issue came to the forefront when Craig Venter, then from NIH, and colleagues applied for a patent on recently discovered ESTs. In the first review of the application the patent office rejected all the claims for failure to meet the criteria of utility, novelty, and non-obviousness. The ESTs do not specifically define gene function but they provide information for isolation of the entire gene and for determining the gene location in relationship to QTL. Considerable patent case law exists now exists which

relates to their utility, non-obviousness, and enablement (*Nebel et al.,* 2002). The patent office has decided ESTs are patentable if it can be shown that they are useful, but if the patent does not claim the entire gene sequence it has limited economic value. Companies, like Incyte Pharmaceuticals, have protected these ESTs by creating proprietary databases that are useful in predicting gene function and in the development of medical and veterinary technologies.

Patent coverage is not just confined to genetic markers. Lines of pigs or chickens have been patented. Other patents exist for methods involving cellular and animal manipulation and involve processes like stem cell development, transgenic production (e.g., US 6,271,436) and cloning (e.g., US 6,215,041 or US 6,258,998). Several advances related to mechanical or electronic devises have been made and include new A.I. tools, advanced ultra-sound equipment, formulas and methods to measure back fat and other traits in livestock (e.g., see early patent US 4,359,055 and more recent US 5,717,142). The need for traceability of animals and animal products has spawned a number of inventions including electronic ID tags and retinal scanning methods and devices.

Considerable discussion has ensued recently from a patent entitled "Method of Bovine Herd Management" granted in the US in 1994 and later in Canada (*Schaeffer,* 2002). The invention is for the "test-day model" and includes the gathering, mathematical treatment, and the use of the modified data by dairy producers. The novelty and unobviousness of the patent has been seriously questioned. It was pointed out that the practices of gathering, manipulating and using data by dairy producers have existed for nearly 100 years. The patent therefore claims rights to a practice that has been public knowledge for a long time. The novel idea within the patent was the specific mathematical model and procedures that Everett and co-workers developed for the analysis of test day yields. Everett was also not the first researcher to apply a model to test day records and as has been demonstrated, the model as described in the patent, is not necessarily the best model that could be applied (*Schaeffer,* 2002). This patent generated considerable discussion. It has been argued "what would the field of animal breeding be if the selection index or Henderson's BLUP had been patented?" Yet while quantitative geneticists see the thought of such protection as sacrilege, molecular scientists accept (but may not like) that in a similar way the foundation patent for PCR exists and royalties must be paid for its use.

In the US and some other countries, there is no clear exception to infringement for research use of patented inventions, even in non-profit or educational institutions. Researchers are required to obtain a license or permission from the patent owner to use a protected invention for research purposes. More and more universities are enforcing this practice. In other countries, there is either an exemption for educational or non-commercial research or research on improving the invention. Researchers should also be concerned with possible claims for "contributory infringement," i.e., assisting someone else to infringe on a patent. For example, due to a claim of possible contributory infringement, the US Genome Coordinators were asked to halt the practice of supplying primers for microsatellite analysis to other researchers. To resolve the issue the Coordinators began buying the primers from a company with a research license.

Ethical, social and economic issues

Many ethical and social issues have been raised related to patenting of animals and genes (*Evans*, 2002). These include: 1) patenting of animals or genes will be destructive to nature and allows man to play "God"; 2) patenting will devalue animal life and hence human life; 3) patenting will increase animal suffering; 4) patenting will lead to a decline in genetic diversity of animals and threaten species; 5) patenting speeds the trend toward commercialisation of academic research; and 6) patenting will undermine conventional farming and lead to increased industrial farming systems. Early humans domesticated animals and master breeders and geneticists have transformed them into productive species. Was this playing "God" or interfering with nature? The use of transgenics for making specialized animal lines for disease research is certainly adapting nature but does it devalue life and is it unethical or immoral? These are value judgments that most in society have decided are acceptable. Certainly some lines have been (and need to be) drawn to delineate what is acceptable and unacceptable. For example, most people and governments have concluded that while animal cloning is acceptable (at least for research purposes), cloning of humans, for whatever reason, is immoral, unethical and to be avoided.

Animal welfare and animal rights issues continue to be at the forefront of livestock production and biomedical research (*Evans*, 2002). Individuals who believe that animals have "rights" will likely be opposed to patenting any invention derived from animal research. The most commonly cited examples are those relating to transgenic animals (e.g., early transgenic pigs) in which some animals had health problems. Production and patenting of specialized lines of rodents for biomedical research that have a tendency to develop specific diseases is also considered unethical by animal rights activists. If, however, individuals believe that animal rights are subordinate to those of humans, but that they deserve proper care and welfare then the issue of patenting is much less of a concern.

It has been suggested that through the use of gene markers and highly selective breeding, or through the use of transgenics and cloning, genetic diversity will be greatly reduced. Certainly these methods have the potential to remove within-line variation but they are likely to increase between-line variability. However, it may be argued that the patent system in fact encourages diversity as it promotes and helps establish, via patent-related deposits of biological materials, a broader genetic diversity. Related to this area is the issue of patenting of products from animals or plants from developing countries. Should companies be allowed to sample and alter wild stocks and to subsequently earn great sums of money by then selling them to both developed countries and back to the countries from where they were obtained? Some individuals say this so-called biopiracy is encouraged by patenting and some countries are moving to address these concerns (*Evans*, 2002). An extension of this is the issue of species integrity. The development of transgenic pigs for xenotransplantation and other species with human genes for human protein production threatens the genetic differences that determine species. While consideration of xenotransplantation has slowed due to fears of retroviruses and diseases like mad cow disease and AIDS, as *Evans* (2002) points out this dispute over species integrity "is not em-

pirical" but is tied up in the individual and societal views on nature and "the notion of awe and wonder." No matter how repugnant this process is to some, there will be development of transgenic animals for biomedical research and applications that do encompass genes from other species.

In a perfect world, public support of research would be 100% of required funding and all IP would be publicly available in the country of origin. Many US public institutions are now funded at about 40% of total budgets and pressures to obtain outside funding are growing. Companies supplying funding expect to "own," through licensing agreements or otherwise, the IP that results. Protection of IP can provide research partners a greater basis for trust and exchange of ideas and help insure that public institutions focus research on areas of high relevance and payoff. Commercial relationships also aid in technology transfer, employment opportunities for students and may allow for research opportunities not available in the public sector. *Fretz and MacKenzie* (2002) have suggested that: 1) such activities serve the public good; 2) short term, low quality research should not be favoured in order to obtain funding; 3) managing of IP must be a balance of serving the institution, funding agency and public; 4) independence of the researcher and institution must be maintained; 5) the mission of the public institution is not altered; and 6) conflicts of interest should be avoided. Institutions must be proactive in balancing funding, IP protection and commercialisation.

Does patenting increase the likelihood of "industrial farming?" Economic and governmental issues certainly play a role in the size of the average farm and the level of commercialisation of farming. It can be argued that if large companies have exclusive licensing arrangements for genetic tools then small breeders will be disadvantaged. Market pressures related to size of operation and efficiency of production are much larger influences on the industrialization of farming and livestock production than patenting.

Langinier and Moschini (2002) provide an excellent review of the economics of patenting and the benefits and costs that are derived. While inventors would prefer broader claims, limited claims encourage competition and further innovation. Complex products often require building on other patents. Unfortunately, new development can be blocked by other patents (especially at the leading edge) and this has negative effects on both the developer as well as the public. Licensing of patents exclusively to one company may benefit that company and segments of the public but might also limit further innovation and not be in all the public's best interest. Additional efforts are required to improve the workings of the patent system to improve the economic performance of the overall system.

CONCLUSIONS

In the 21st century sequenced genomes, transgenic livestock and cloned animals will become the norm. These discoveries and their uses represent the intellectual property of individuals and teams. As pointed out in the opening quote of Bruce Lehman, former US Commissioner of Patents and Trademarks, "The only wealth there is in the world is the wealth that comes from the human

mind." Animal breeders have begun to patent their IP and this has raised economic, legal and ethical concerns that might affect the support of public education and research. Patents do not block the spread of knowledge but instead can aid technology transfer. The "landscape" of agriculture has changed with increased vertical integration of production and there is more control from the farm to the consumer's table. Inventions move more quickly into the market place but certain production sectors may be disadvantaged. While the public is concerned about the safety of products and access to them, it must be assured that patenting will continue to promote progress and not prevent it. Patent applications must not be frivolous and the real costs of patenting must be reasonable. A reasonable percentage of profits from patenting must be reinvested into research to promote future discovery. Finally, scientists must work to see that the IP that is produced has usefulness, real value, does not harm animals or humans and promotes the public good.

ACKNOWLEDGEMENTS

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SUMMARY

Animal breeding and genetics changed markedly in the 20th century and changes in the 21st century should likely be larger with sequenced genomes, transgenic livestock and cloned animals becoming the norm. These discoveries and their uses represent the intellectual property or "inventive" work of individuals. Animal breeders in universities and in governmental research labs have begun to protect their inventive works through patenting. Patents and their implications are discussed in detail. Concerns by the public about economic, legal and ethical issues are presented. It is concluded that patents do not prevent the spread of knowledge but have aided technology transfer. Patent applications must not be frivolous and the real costs of patenting must be reasonable. Profits from patenting must be reinvested into the research system to promote future discovery. Scientists and inventors must work to see that inventions that are produced have usefulness for society, do not harm animals or humans and do the most to promote the public good.

A GÉNTARTALÉKOK VÉDELME ÉS AZ INFORMÁCIÓK SZABAD CSERÉJE: A SZABADALMAZÁS SZEREPE A TENYÉSZTÉSBEN ÉS GENETIKÁBAN

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ÖSSZEFOGLALÁS

Az állattenyésztés és a genetika jelentősen megváltozott a 20.században, és a 21. századi változások még nagyobbak lesznek, mivel a szekventált génállomány, a transzgenetikus haszonállatok és a klónozott állatok megszokottá válnak. Ezek a felfedezések, valamint ezek alkalmazása, felfedezőjük szellemi terméke és tulajdonát képezik. Az egyetemek és a kormányzati és egyéb kutatóintézetek állattenyésztői, már megkezdték a nyilvánosság azokkal kapcsolatos saját szellemi termékeik szabadalmaztatását. Az előadás a szabadalmakat és azok alkalmazását részletezi, valamint gazdasági, jogi és erkölcsi fenntartásait tárgyalja. A végkövetkeztetés, hogy a szabadalmak nem akadályozzák meg a tudás terjedését, de segítettek a technológiák elterjesztésében. Nem szabad, hogy a szabadalmakat megfontolatlanul alkalmazzák, és a szabadalmaztatás költségeinek ésszerűnek kell lenni. A szabadalmaztatásból származó profitot vissza kell forgatni a kutatómunkába, hogy az a jövőbeli felfedezéseket segítse. A tudósoknak és feltalálóknak úgy kell tevékenykedniük, hogy felfedezéseik hasznosak legyenek a társadalom számára, ne legyenek ártalmasak az állatok és emberek egészségére, és meg kell mindent tenniük, hogy a közjavát szolgálják.

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CROPS DERIVED FROM AGRICULTURAL BIOTECHNOLOGY AS ANIMAL FEEDS: BIOLOGICAL, ECONOMIC AND SOCIAL RISKS

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INTRODUCTION

Crops derived from agricultural biotechnology were introduced beginning in the mid-1990s after extensive regulatory review. They have been used widely in commercial agriculture, particularly in North America and Argentina. The first crops derived from agricultural biotechnology have improved agronomic characteristics. This involved either of the following:

The expression of the *Bacillus thuringiensis* (*Bt*) transgene encoding the δ-endotoxin CryIA in corn/maize or cotton and thus reducing Leptdopteran insect damage, insecticide usage and equipment/diesel/labor costs as the number of insecticide applications were reduced.

Tolerance to herbicides such that a broad-spectrum herbicide could be used and the growing crop protected from weeds and the herbicide. The first was glyphosate tolerant [e.g. Round-Up Ready® soybeans, canola/oil seed rape, sugar beet], due to the presence of the transgene *epsps* (either a bacterial, or engineered plant gene). More recently crops tolerant to glufosinate ammonium (butanoic acid, 2-amino-4-(hydroxymethylphosphinyl)-monoammonium salt) (marketed as Basta®, Liberty®) have been developed with the transgene

(*pat*) expressing the enzyme — phosphoinothricin acetyl transferase which inactivate the herbicide.

Other commercialised crops derived from agricultural biotechnology include virus resistant papaya and the "flavr savr" tomato (approved by the US Food and Drug Administration (FDA) in 1994). Worldwide plantings of crops derived from agricultural biotechnology for 2001 were in million hectares — 33.3 herbicide tolerant soybeans, 9.8 corn/maize (e.g. *Bt* corn/maize), 6.8 cotton (e.g. *Bt* cotton) and 2.7 canola/oil seed rape (e.g. Herbicide tolerant canola) (from ISAAA). New crops derived from agricultural biotechnology will likely include tolerance to environmental stresses (e.g. low availability of water); improved nutritional qualities; plants for biorenewables; removal of allergens from food ingredients and transgenic animals. This paper addresses the controversy about crops derived from agricultural biotechnology and their safety.

Terminology

The FDA considers that the term genetically modified or (GM) or genetically modified organism (GMO) is misleading and potentially confusing to consumers. *"Genetic modification" means the alteration of the genotype of a plant using any technique, new or traditional. "Modification" has a broad context that means the alteration in the composition of food that results from adding, deleting, or changing hereditary traits, irrespective of the method. The term "GMO free" may be misleading on most foods, because most foods do not contain organisms (seeds and foods like yoghurt that contain microorganisms are exceptions). It would likely be misleading to suggest that a food that ordinarily would not contain entire "organisms" is "organism free."* The potential for confusion comes from the obvious point that geneticists have long improved agricultural crops and livestock using inherent genetic differences between individuals/populations. In addition, plant geneticists have increased these genetic differences using chemical mutagens and radiation. The latter induced changes range from point mutations to major changes in control elements to deletions of a single gene or series of genes. The advent of agricultural biotechnology (in plants, animals, bacteria etc.) has brought the ability to add or delete a specific gene or series of genes and to use gene markers and candidate genes to facilitate rapidly improved selection. Thus, depending on the approach used, the resulting plants may or may not contain a transgene(s). The commercial application of agricultural biotechnology to crops has been predominantly transgenic plants with a bacterial gene inserted. This paper will use the term crops derived from agricultural biotechnology, although the term bioengineering could also be used.

Safety issues with crops derived from agricultural biotechnology

In the USA, crops derived from agricultural biotechnology are regulated by three agencies of the Federal government, namely the FDA, the Environmental Protection Agency (EPA) and the United States Department of Agriculture (USDA). Regulatory approval is evidence of safety or lack of risk (reviewed *Faust*, 2002). The FDA regulates the introduction of new foods and food ingre-

dients for safety and wholesomeness under the Federal Food, Drug and Cosmetics Act. The FDA evaluates the product for safety, allergenicity and toxicity. This consists of a series of questions that have to be answered by the developer of the crop with well-documented research. Approval is when the FDA has no further questions. Submission of agricultural biotechnology to FDA is "voluntary" but every commercial product from biotechnology (plant varieties, vaccines etc.) has undergone full regulatory approval by Federal agencies. The rationale for this is liability protection of the company. It might be noted that the FDA is considering moving to a mandatory system (*USFDA*, 2001). The USDA (the Animal and Plant Inspection Service, APHIS) regulates field-testing of crops derived from agricultural biotechnology. The EPA regulates agricultural biotechnology under the Federal Insecticide, Fungicide and Rodenticide Act and the Toxic Substances Act when there is bioengineering of an insecticide etc. as with Bt maize/corn.

The framework for approval of crops derived from agricultural biotechnology is based on the following precepts. Regulation is of the safety of the product not the process (although environmental and manufacturing safety has to be assured). Crops derived from biotechnology are not fundamentally different from traditionally produced crops and pose no different risk from those modified by classic genetic methods (e.g. *US GAO*, 2002). The FDA stated that they regulate new plant varieties produced through biotechnology in the same fashion as those derived by traditional methods (*US FDA*, 1992).

There is abundant evidence for the safety of crops derived from agricultural biotechnology either directly as part of the human diet or indirectly via feeding to livestock. At present most transgenic crops are fed to livestock. The proteins expressed in presently approved transgenic crops exhibit very low toxicities. In acute toxicity testing for safety, the no observed effect level (NOEL) for the Cry proteins (CryIAb, CryIAc, CryIIAa, CryIIAb, CryIIIA) was ~4g per kg body weight in higher vertebrates (reviewed *Faust and Glenn*, 2002). Similarly, the NOEL for 5-enolpyruvylshikimaye-3-phosphate synthesis (EPSPS) in Round Up Ready® crops was ~0.6 grams per kg body weight in higher vertebrates (reviewed *Faust and Glenn*, 2002). It may be questioned whether grain from transgenic crops has the same composition as traditional crops. No differences are found. Were there to be differences, the US regulatory position is that the transgenic crop is not compositionally comparable (i.e. not substantially equivalent) and a different regulatory paradigm is triggered.

Potential for allergenicity in crops derived from agricultural biotechnology

There are concerns lest the products of expressed transgenes exhibit allergenicity. A decision tree has been developed to screen out proteins with high probability of allergenicity (*FAO/WHO*, 2000; reviewed *Kleter and Kuiper*, 2002).

Is the source of the gene allergenic? If yes and exhibits sequence homology to known allergen, then allergenicity is likely.

If no sequence homology, use specific serum screen to test.

If there is a positive in the test, then allergenicity is likely.

If negative, go to targeted serum screen. If positive, then allergenicity is likely.

If negative determine resistance to pepsin digestion and allergenicity in animal models. If resistant to pepsin and evidence for allergenicity in model, then human allergenicity is likely.

Only products of transgenes where the probability of allergenicity is low are developed further.

Environmental safety of crops derived from agricultural biotechnology

It is also essential that products of food/agricultural biotechnology are safe to the environment. Wildlife, for instance birds, may consume these crops both directly and through feeding on insects, etc. that have eaten the crops derived from agricultural biotechnology. When examining the impact of crops derived from agricultural biotechnology on the environment, it is important that valid comparisons are made. For instance the complete agronomic system for crops derived from agricultural biotechnology together with herbicide/pesticide regimen should be compared to an alternative system (including requisite herbicide/pesticide regimen).

Performance of livestock fed crops derived from agricultural biotechnology

There is now a considerable body of evidence that livestock fed on grain from biotechnology-derived crops show similar performance to that from conventional crops (reviewed *Clark and Ipharraguerre*, 2001; *Faust,* 2002; *Faust and Glenn*, 2002). One of the first and most thorough published studies examined the effects of *Bt* corn on broiler chickens (*Brake and Vlachos*, 1998). Growth rate was not affected. An improved feed-to-gain ratio in chickens was reported (*Brake and Vlachos*, 1998). This may have been due to the *Bt* corn having reduced levels of mycotoxins (e.g. *Munkvold et al.,* 1999). Fungal toxins or mycotoxins are produced by mold infestation of grains prior to and following harvesting. It should be noted that no effect on broiler chicken growth or feed:gain was observed with *Bt* corn produced from a different transformation event (*Brake et al.,* 2003). This may reflect differences in corn borer prevalence and hence mold infestation. There is anecdotal evidence from local farmers that pigs fed *Bt* corn show an increase in reproductive problems, specifically pseudopregnancy. A team of veterinarians and toxicologists investigated this. No specific association of reproductive problems and the feeding of *Bt* corn was observed (*Carr et al.,* 2003). Moreover, the *Bt* corn had no detectable estrogenic activity.

Safety of animal products from livestock fed grain from agricultural biotechnology crops.

Crops derived by agricultural biotechnology undergo review by US Federal agencies (see above). New crops must now satisfy review as both animal feeds and as human food before commercialisation. There is no evidence to suggest that milk, eggs or meat from livestock fed grain from crops derived from agricultural biotechnology have any changes in their composition or represent any threat to human health (reviewed *Clark and Ipharraguerre*, 2001; *Faust,* 2002; *Faust and Glenn*, 2002). In Europe, chymosin is used extensively in cheese making. For instance, in the United Kingdom, 90% of cheese is produced using

this bacterially produced enzyme. It has been argued that the cheese is "GMO-free" as the enzyme is degraded during cheese maturation and that this is the product of a "GMO" not the organism itself (*http://www.ncbe.reading.ac.uk/NCBE/GMFOOD/chymosin.htm*). The same argument can be made for livestock products from animals fed grain from crops derived from agricultural biotechnology, vaccines for livestock and poultry and bovine somatotropin (bST) in milk production or even oil from *Bt* corn or Round Up Ready® soy beans. Indeed, the FDA states: "*For many foods, however, particularly for highly processed foods such as oils, It may be difficult to differentiate by validated analytical methods between bioengineered foods and food ingredients and those obtained using traditional breeding methods*".

Is it possible that the transgene from transformed crops could be found in livestock products (milk, meat or eggs) then consumed by people? On the face of it, this seems to be unlikely as animal intestines have been exposed daily to foreign DNA for billions of years. Both the United Nation's Food and Agriculture Organization and the US Food and Drug Administration concluded in 1991 that consumption of DNA posed no health risk (US FDA, 1992). Moreover, no *cry*1Ab genes were detected in tissues from cattle or chickens fed Bt corn (*Einspanier*, 2001; *Klotz et al.*, 2001). Similarly, in studies with dairy cows fed silage made with Bt corn, no fragments of the *cry*1Ab genes were detected in the milk (*Faust*, 2000; reviewed *Faust and Glenn*, 2002). There is also strong evident that the *EPSPS* gene is not detected in milk of dairy cows even when receiving a diet containing 26% herbicide tolerant soybeans (*Phipps, et al.*, 2002). Given the very low content of *cry* genes etc. in grain, the possible transfer of genes is extremely unlikely (*Beever and Kemp*, 2000).

There is no evidence for plant genes being incorporated into animal genomes as would be expected were there to be germline transfer of genes from food into the animal or human. However, there is some preliminary evidence for plant/chloroplast DNA fragments in cattle lymphocytes and small intestine and chicken muscle, spleen, kidney and liver (reviewed *Aumaitre et al.*, 2001). Other workers have been unable to detect similar plant DNA fragments when using asceptic laboratory techniques (*Klaften et al.*, 2003).

The controversy about crops derived from agricultural biotechnology

Biotechnology has applications to medicine, agriculture etc. While the general public rapidly accepted biomedical biotechnology, there has been significant opposition to agricultural biotechnology. Agricultural biotechnology was the first new technology that was not driven by consumers and there was not a public debate initially on agricultural biotechnology. Another factor to consider is that the first commodity crops derived from agricultural biotechnology provided no discernible benefit to consumers coupled with perceived risks.

Much of the earlier discussion on crops derived from agricultural biotechnology stems from Western Europe. There has been considerable reluctance from the European Union to allow the import of North American grain. Some in the USA contend that the issue is solely one of interference with trade (*i.e.* a non-tariff barrier to trade). However, it is argued this is an over-simplification. The coming of agricultural biotechnology involved or was perceived in Europe to ·

involve a product "pushed" by a single large American chemical company and, as a result, consumers were offered no choice. This led to resentment across the political spectrum. Moreover, the major crops involved (corn, cotton and soybeans) are not strongly historically or culturally linked to Europe. There was not sufficient involvement of other companies, regulators or university scientists in the discussion. It might also be indicated that there was initially relatively little third party testing of crops derived from agricultural biotechnology and refereed publications. An additional important factor was the impact of diseases such as BSE (bovine Spongiform encephalitis and foot and mouth disease) which eroded public confidence in the scientific establishment and in science based regulatory systems in Europe. Moreover, the Common Agricultural Program of the European Union essentially "de-links" food prices from agricultural production. Thus any decrease in the cost of production is not reflected in a change in food prices and provides no direct benefit to consumers.

Agricultural researchers have done an outstanding job in improving the yield of crops and the rate of production of livestock together with ensuring a pleasing appearance to the food (bright red apples without blemishes, large red strawberries etc.). Moreover, they are now addressing the nutritional qualities of food. However, there has been generally a lack of attention to the eating quality or gustatory characteristics of food (flavour, texture etc.) perhaps with the meat industry being an exception. Agricultural biotechnology could have an impact here, if markets were to be willing to pay for specific characteristics. It appears that many retailers have not yet appreciated a value to enhanced "eating" characteristics. The inattention to eating quality, coupled with globalisation and the perception of a lack of sensitivity to the culture of regions, counties or districts, has resulted in a thoughtful support of traditional systems and to organic production systems. However, the economics of these may be only viable with very substantial government subsidies and/or consumers paying considerably higher prices. There is likely to be increases in niche markets ranging from organic, genetic modified organism (GMO) free, natural, family farm produced etc. Given a free market and consumer choice, there will be increasing segmented markets for fruits, vegetables and livestock products, particularly in the most developed/wealthiest countries.

No one can argue that crops derived from agricultural biotechnology should not go through rigorous regulatory approval with demonstration of safety to people (including allergenicity), livestock and the environment together with efficacy. These issues are discussed in detail. It is urged that sound science should be the basis of public policy as outlined in the series of FAO/WHO Expert Consultations on Foods derived from Biotechnology and discussions of the Intergovernmental Codex Task Force (*Eno and Boutrif*, 2002). Moreover, there is a strong case for harmonization of the methodologies employed in different countries. Moreover, scientists should be part of the public dialogue.

The European Union will require labels for foods and animal feeds produced from crops derived from agricultural biotechnology but not of livestock products raised on these crops. The position of the US government is to require labelling only where there is a public health issue (e.g. concentrations of fat and of saturated fat in foods, potential allergenicity or altered nutritional characteristics). Moreover, labelling potentially creates the perception of a safety problem

or of inferiority of the product. It might also be noted that where labelling had been practiced, as in the case of the "flavr savr" tomato in tomato paste in the U.K. market, grocery stores were successfully pressured to remove it by activists.

Risks of not adopting crops or livestock derived from agricultural biotechnology

Potential risks for adopting biotechnology derived crops are cited and discussed frequently, however societal risks may exists for those who reject this technology and its potential benefits. In fact, *Kershen* (2000) cites three primary risks to food producing companies and society in general that can result when no substantial risks are identified and biotechnology derived crop which are superior to conventional crops are not used. Risks identified by *Kershen* (2000) include the following: product liability and environmental compliance.

It is argued that food-producing companies that do not allow the use of biotechnology derived crops may be liable when consumers contract diseases that may have been prevented by using these crops. Similarly, growers and their employees who are harmed by agricultural practices such as the use of certain organophosphate pesticides which are unnecessary when specific biotechnology derived crops are adopted — e.g. insect-resistant plants — may be able to bring law suits against food/feed purchasers who restricted the planting of these crops. When biotechnology derived crops with environmental benefits, such as low phytic acid corn are disallowed, food producers may be held partially responsible for the negative environmental impacts. In an analogous manner, there are moral consequences and political ramifications if countries or supranational. *Kershen* (2000) concluded that, "*the decision to adopt scientific ignorance about agricultural biotechnology may be unsustainable, politically and morally, on our globe*" (*Kershen*, 2000).

CONCLUSIONS

A strong case is made for using sound science as the basis of public policy when ever possible. The United Nations Food and Agriculture Organization (FAO) estimates that there are 840 million people in the world who do not receive enough to eat. It is undeniable that the pressures against agricultural biotechnology are slowing the application of biotechnology to crops of developing countries where the vast majority of undernourished people live. Recently *Qaim and Zilberman* (2003) reported yield improvements of ~87% in Bt cotton in India. There is an overwhelming case that we as a global society should to use all the tools of the agricultural sciences to overcome nutritional deprivation, malnutrition and to replace fossil fuel with biobased products. *Conway and Toenniessen* (2003) have provided a compelling case that world food and fibre production can be markedly increased by both improving soil fertility and employing improved crops varieties (greater yield potential + resilience to weeds, insects and drought) developed through traditional means and biotechnology.

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SUMMARY

The use of crops derived from agricultural biotechnology and their application as feeds to live-stock is discussed. The regulatory system employed in the USA is described. The literature of the safety of crops derived from agricultural biotechnology is summarized, as are the effects of grain from these on livestock performance. Policy issues such as the use of sound science as the basis for regulation and that of labelling products from agricultural biotechnology are addressed. The development of niche markets is considered. In addition, the potential use of molecular techniques directly to livestock is addressed.

NÖVÉNYI TAKARMÁNYOK A MEZŐGAZDASÁGI BIOTECHNOLÓGIÁBÓL: BIOLÓGIAI, ÖKONÓMIAI ÉS TÁRSADALMI KOCKÁZATOK

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ÖSSZEFOGLALÁS

Az előadás a mezőgazdasági biotechnológia alkalmazásával előállított termények felhasz-nálását és alkalmazását a takarmányozásban ismerteti. Bemutatja az Egyesült Államokban alkalmazott szabályozó rendszert. Az előadó összegzi, az így előállított termények biztonságával kapcsolatos irodalmat, valamint tárgyalja ezek hatásait az állatok teljesítményére. Olyan kérdéseket is megvitat, mint a törvényi szabályozás alapján álló korrekt tudományos eredmények alkalmazása, valamint a biotechnológia segítségével előállított termékek jelölése. Ezeken kívül, foglalkozik még a molekuláris technikák közvetlen, haszonállatokon való potenciális alkalmazásával is.

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Meyn Klaus, 1937-ben született Észak-Németországban. 1964-ben mezőgazdasági diplomát szerzett a göttingeni egyetemen és a kelet-afrikai üzemi gyakorlatok után, 1967-ben kapta meg a doktori címet. Meyn a Washington D.C.-ben működő Világbanknál dolgozott, mint haszonállat specialista, majd a Német Állattenyésztők Szövetségét illetve a Szarvasmarha-tenyésztők Szövetségét vezette Bonnban. 2002-ben ment nyugdíjba ebből a beosztásból. 1990–1994-ig az ICAR, 1991–1996-ig az EAAP közép- és kelet-európai külön bizottságának elnöke volt. Tagja a Svéd, az Orosz és az Ukrán Mezőgazdasági és Erdészeti Akadémiáknak, 1998-ban elnyerte az EAAP Leroy díját, 2002-ben a Göttingeni Egyetem díszdoktorává avatták.

ANIMAL PRODUCTION STRATEGIES FOR THE FUTURE

MEYN, KLAUS

INTRODUCTION

Differing animal production strategies are possible at global, regional or national levels, and these strategies may be in conflict with each other. Thus, a global strategy by the World Trade Organisation (WTO) or the World Food Organisation(FAO) may look different to, or run into conflict with, the strategy of the European Union(EU), and this, in turn, may differ from the animal production strategy of Hungary, because in each case the strategy should achieve the set goals.

While the main issues at global level are malnutrition and the improvement of diets through a higher proportion of animal protein, the developed World is facing conflicts between countries with low production cost pastoral industries in the New World and countries in the northern hemisphere with high cost subsidised production (*Table 1*).

Table 1.

Low and high cost animal production countries

Low cost pastoral	High cost subsidised
New Zealand	Japan
Australia	Norway
Argentina	EU
Uruguay	USA (dairy)
Chile	
USA (beef)	

Furthermore, countries with cheap feed supplies are beginning to exploit the export opportunities for poultry meat, pork, milk and dairy products, and some rich countries have a large deficit in animal products and are the main importers of animal products (*Table 2*).

Table 2.

Main exporters and importers of poultry meat and pork

Exporters	Importers
USA	Russia
Brazil	Hong Kong
Canada	Japan
EU	China
Thailand	Saudi Arabia
Hungary	
Poland	

Sources: ZMP(2002abc)

The animal industries of Central and Eastern Europe (CEE) are in a special situation. Since the collapse of communism they have not yet been able to return to stability. During the first years after the political and economic changes they tried to come to grips with the farm structures leading to declining production. At the same time the falling purchasing power of the consumers led to lower producer prices. Furthermore, export markets collapsed and new ones had to be found. Currently they are forming three groups of countries: the twelve members of the Commonwealth of Independent States (CIS), the ten accession countries that will join the EU in 2004, and the seven Balkan states that will or may join the EU at a later date (*Table 9*).

Development of food supplies of animal origin and animal production policies

World supplies of food of animal origin: At global level, the four decades from 1960 to 2000 have seen great successes in solving the World food problem. Food balances available between 1962 and 1988 prove that the calorie requirements of the people, especially in China, were improved substantially. But also the luxury consumption of calories from animal origin in the USA, Australia, and to a lesser degree in Europe, may have caused health problems so that their proportion in the diet was reduced (*Table 3*), while the proportion of animal protein was maintained (*Table 4*).

From 1980 to 1999, World food supplies received a further boost in every continent except Africa, both in terms of total and per capita production, despite a global population increase of 36%. For people in Asia the supply with food of animal origin was more than doubled (*Table 5*). China succeeded to increase the proportion of animal protein in the diet by 11%, i.e., from 8 to 19% (*Table 4*), and information on diets available from Hong Kong and Singapore confirms that with rising living standards Asians will increase their consumption levels of animal protein to those of the western World. In order to bring about this improvement in human nutrition, a substantial increase in World animal production was necessary. During the 29-year period from 1970 to 1999, World production of poultry meat, eggs and pork more than doubled, while the production of beef,

milk, mutton and lamb increased by only just over one third, and did not keep pace with population growth (*Table 6*).

Table 3.

Per capita supplies of calories from 1962 to 1988
(1961–1963=100)

Region	Daily calorie supply	Calorie index	% calories of animal origin	% change from 1961–1963
World	2,703	118	16	0
Africa	2,360	113	8	0
South America	2,674	112	18	0
Asia	2,487	132	9	+3
China	2,634	158	10	+6
India	2,196	110	7	+2
Europe	3,459	112	33	+5
USA	3,676	115	34	–6
Australia	3,186	104	36	–7

Source: FAO Yearbooks Production, Rome, 1961 to 1989

Table 4.

% animal protein in total human protein consumption, 1962 to 1988

Region	1961–1963	1987–1989	Change of % points
World	32	35	+3
Africa	21	21	0
South America	42	45	+3
Asia	15	22	+7
China	8	19	+11
India	10	14	+4
Europe	48	58	+10
USA	68	67	-1
Australia	67	67	0

Source: FAO Yearbooks Production, Rome, 1961–1989

Table 5.

Indices of population, food production and food of animal origin, 1999
(1979–1981=100)

Region	Population index	Food production index	Index of food from animal origin
World	136	150	147
Africa	161	160	155
South America	141	173	166
Asia	138	202	277
Europe	105	107	102
USA	121	127	127
Australia	127	149	124

Source: FAO Yearbooks Production, Rome, 1979–1999

It was Asia again with its more than 60% of the World's people that succeeded in achieving the highest production increases, while Europe, restricted by production quotas in the west and political and economic changes in the east showed the slowest development in animal production (*Table 7*).

Table 6.

World indices of food production of animal origin, 1999

Product	Index (1969–1971=100)
Eggs	254
Meat	213
poultry meat	374
pork	228
beef .	144
mutton and lamb	132
Milk	138

Source: FAO Yearbook Production, Rome, 1969–1999

Table 7.

Regional indices of food production of animal origin, 1999
(1969–1971=100)

Region	Meat	Milk	Eggs
World	213	138	254
Africa	206	208	370
South America	433	240	327
Asia	395	299	497
Europe	161	105	117
USA	163	138	119
Australia	174	132	104

Source: FAO Yearbook Production, Rome, 1969–1999

EU: Food security and the provision of adequate incomes to farmers were the main goals of the Common Agricultural Policy (CAP) of the EU emerging during the nineteen sixties, originally with six member states, but eventually binding together 15 countries after four enlargements, and forming the most powerful consumer market in the World.

The price incentives to producers were so successful that surpluses occurred in milk, beef, grains, oilseeds and sugar (*Table 8*). Consequently, the CAP had to be modified, in order to restrict production, to reduce export surpluses and to allow easier access of agricultural goods from the World market. In three major steps in 1984, 1992 and 2000 milk quotas, animal premiums and set-aside programmes were introduced, and producer price levels were brought closer to World market levels. At the same time the collapse of communism reduced the risk of war, and the food security problem had lost its threatening importance. The "Uruguay-Round" of the GATT during the nineteen nineties and the current "Doha-Round" of the World Trade Organisation have moved the EU closer to an open market, but some issues such as the types and levels of subsidy still allowed and the permission or prohibition of hormones and genetically modified organisms are not yet resolved. In addition, the pending enlargement of the EU by eight CEE and two Mediterranean countries in 2004 and the eventual accession of more Balkan countries poses problems on the financial sustainability of the CAP.

CEE and CIS countries: Dramatic changes have happened to the agricultural sectors of the CEE and CIS countries following the political changes from 1989 to 1991. Earlier, collective or state farms were the rule in all these coun- .

tries except Poland and former Yugoslavia. Four types of development occurred during the changes:

— Smallholder structures were maintained where they existed because of the need for subsistence and as social buffer;

— Large and small farms emerged side by side in relatively advanced countries such as Hungary, the Czech Republic, the Slovak Republic (and East Germany);

— Collective farms were destroyed in the three Baltic countries, Albania, Bulgaria and Romania leaving behind a large number of subsistence households;

— Large farms in the CIS countries were converted to farming companies or cooperatives, but due to management problems on most of them a strong household sector recruited from former farm workers emerged. For example, subsistence households have contributed more than 56 % to animal production in Russia in 2001 (*ZMP*, 2002c).

Table 8.

Self sufficiency levels of animal production in the EU, 2000

Product	%
Meat	
beef and veal	104
pork	109
poultry meat	107
mutton and goat	81
Milk	
without subsidy	117
with subsidy	108
Eggs	102

Source: ZMP(2002abd)

Animal production in countries with traditional or new smallholders has proven to be stronger than in the countries with an important large farm sector (*Table 9*), in part, because low producer prices would not have justified production, but the subsistence function contributed to stabilise the sector. In recent years, subsidies in some accession countries and strong market demand in the population centres of Russia have helped to revive the industry.

Future strategies for animal production

Goals: An appropriate animal production strategy should be a comprehensive approach to the further development of the sector including the exploitation of market opportunities and perspectives, marketing and processing facilities, production potential and restrictions, expected technical progress, feed conversion efficiency of the different animal species, and availability of the production factors land, labour, management and capital.

Market perspectives: First, local market perspectives should be screened. In the case of Hungary, the expected rise of incomes through EU accession will probably lead to increased demand for animal products, and this should be met as a priority.

Index of animal production in CEE and CIS countries 2000
(1989–1991=100)

Country group	Population Mill.	Animal production index
1. Former small-holder countries	61.9	80
2. Large farm and small-holder countries	25.9	66
3. New small-holder countries	41.2	84.1
4. CIS countries	283.6	58.4

1. Poland, Serbia-Montenegro, Croatia, Bosnia-Herzegovina, Slovenia, Macedonia
2. Czech Republic, Hungary, Slovakia
3. Romania, Bulgaria, Lithuania, Albania, Latvia, Estonia
4. Russia, Ukraine, Uzbekistan, Kazakhstan, Belarus, Azerbaijan, Tajikistan, Georgia, Kyrgyzstan, Turkmenistan, Moldova, Armenia
Source: ZMP (2002c)

But for Hungary as a traditional exporter of animal products the extra opportunities that both the old and the new EU members, the former customers in the CIS and potential new customers elsewhere may offer, must also be studied. As an example, the last recorded net imports of animal products of the existing EU member states are presented in *Table 10*. Vast quantities of animal products are sold by the Netherlands, Denmark, Belgium, Ireland, France and Spain to Italy, the U.K.., Germany, Greece and France. Comparative advantages of Hungary and the other accession states in these markets should be exploited, but the risks associated with the sensitive consumers should not be underestimated. In contrast, the quantities of animal products which are imported by the accession countries are negligible. On the other hand, the economic recovery of the CIS and Balkan countries and recent imports of meat (*Table 11*) and other animal products by these countries should be noted. Whether additional opportunities in the World market could be exploited is open to question, because this requires substantial infrastructure and logistics and should be considered only, if long term contracts for larger quantities may be secured.

On the other hand, the enlarged EU will have a vital interest in reaching the emerging import markets in Asia, the Middle East and North Africa with animal products by setting the appropriate economic framework for EU producers and exporters. Apart from exporting beef and dairy products at subsidised prices, pork from Denmark and specialty cheeses from several EU member states are already successfully placed in the World market. It will be a turning point when China, which has had tremendous economic growth over the past years and which holds more than one fifth of the World's population, will start spending money for animal products imported from the World market. Or will China prefer to import feedstuffs, in order to strengthen its own animal agriculture?

The future is uncertain, but rising living standards will probably increase the demand for quality and specialty products everywhere. For example, Hungarian salami is widely known and should have good chances on a World scale. Equally, specialty cheeses from several EU member states are finding attractive markets in the USA, Russia, Japan, Saudi Arabia and other countries.

Table 10.

Net imports of animal products by the main importing EU member states

	Beef	Pork	Poultry	Mutton and goat	Total
1. Meat, '000 t					
Italy	399	835	—	47	1.281
U.K.	290	689	195	—	1.174
Germany	—	506	572	48	1.126
Greece	164	196	36	23	419
2. Cow milk equivalent, '000 t					
Italy	4,110				
U.K.	3,679				
Greece	675				
3. Eggs, mill.					
Germany	3,536				
Italy	970				
France	954				
U.K.	682				

Source: ZMP(2002abd)

Table 11.

Net imports of meat by selected CIS and Balkan countries 2001, '000 t

	Beef	Pork	Poultry	Total
Russia	322	233	687	1,260
Romania	4	34	28	66
Albania	2	9	24	35
Macedonia	9	6	20	35
Bulgaria	13	7	17	32
Bosnia-Herzegovina	10	8	5	27
Ukraine	—	—	26	26
Croatia	2	21	—	23
Serbia-Montenegro	1	1	13	13

Source: ZMP(2002b)

Unfortunately, there is little chance to provide countries with animal products from the World market which cannot pay for it. Past food aid projects supplying animal products to very poor countries were usually destructive, because they distorted local markets and negatively affected the local animal production sector without bringing sustainable advantages for the consumers.

Marketing, processing and retailing: The concentration in modern retail trade also necessitates higher concentrations in the production, collection, processing and marketing of animal products. Apart from niche markets, there has to be a minimum product concentration to be taken seriously as a market partner. Animal producers must, therefore, be locked into trading and processing organisations with a sizeable output, either through contracts or as shareholders or members of these organisations. Strict quality management programmes of the food chain and cooperation with established organisations in the EU are indicated.

Production potential, geographic location and restrictions: One of the objectives of the formation of the EU is the production of goods in economically optimal locations and their exchange within the Community for optimum welfare.

For example, the grasslands in hill and alpine areas and in areas bordering the North Sea or the Atlantic are natural locations for cheap milk and ruminant meat production. Despite a uniform application of the CAP, average producer milk prices in Italy and Greece were about 20% higher than those in Ireland and the U.K. in 2001 (*ZMP,* 2002b). In areas where maize silage can be grown, the systems can be intensified, and areas where maize can be brought to maturity, excellent conditions exist for fattening beef cattle and pigs, and for keeping poultry. But also other grain growing areas may be suitable for intensive animal agriculture and for competitive production. For example, pig producer prices in Greece and Italy were 57 and 35% higher than in the Netherlands and 40 and 22% higher than in Denmark, respectively in 2001 *(ZMP,* 2002d). On the other hand, the long dry summers in the Mediterranean constitute an expensive environment for keeping dairy animals and meat producing ruminants, but they are quite suitable for poultry and pig production. Hungary, with its potential to grow maize, but with hot summers and relatively cold winters, offers excellent conditions for pig and poultry production, but is not so favourable for dairying.

Geographically it is well placed to three major import markets of the EU: Italy, Germany and Greece.

The price that animal producers in the EU have to pay for relatively stable product prices is production quotas for milk, suckler cows and beef animals and a set of restrictions in animal welfare, hygiene, the application of drugs and medicines, environmental pollution, and the stocking rates of animals. For instance, animal producers joining the EU will have to comply with the quota restrictions laid down in the accession agreement, and any further expansion will include the cost of quota in addition to the investment costs. At national level, there will be upper limits for the quantity of milk that may be sold and the number of suckler cows or male fattening animals, for which premiums may be collected. In view of the steady increase of milk yields per cow, the national number of dairy cows will decline further and this will also reduce the number of male calves born eligible for fattening premiums. The situation is less restrictive with pigs and poultry because they are not subsidised, but the very high standards set for milk, meat and egg hygiene and for environmental protection make it difficult for small producers to survive. In addition, it has become difficult to obtain permits for the new establishment or expansion of intensive livestock enterprises, because of environmental considerations and in order to avoid the irritations of smell to non-agricultural people living in countryside.

Technical progress: Future animal production strategies also must take into account the effects of the ongoing or expected technical progress. While poultry meat production has been industrialised for some time, and capital intensive automation of pig production is underway, the main species of technical innovation appears to be cattle, in particular the dairy cow. Over the last thirty or so years, cattle producers have benefited from the introduction of totally mixed rations (TMR) into their feeding systems, but more recently, the emphasis lies on investments into labour saving and hygienic milking technology. In both cases there are cost savings per cow in larger units, but the new milking technology allows family farmers to increase their herds. As a result, dairy herds are growing fast in size in the United States, Australia, New Zealand and in Europe,

and the number of farms is declining rapidly. Continuous genetic improvement through effective and globally connected breeding programmes exploiting mainly population genetics but increasingly also molecular genetics guarantee that each generation of dairy cows is more productive than the previous one.

On the other hand, there has been virtually no technical development for beef enterprises over the last years. Suckler cow production is an extensive enterprise warranting no intensive. inputs, and the technology of beef cattle fattening has been developed many years ago. Furthermore, the beef industry has suffered heavily from public discussions about BSE during the last decade and, because of competition from cheaper types of meat, it will have difficulties to regain its former position.

Feed conversion efficiency of the different animal species: While ruminants consume a considerable part of their feed in the form of herbage which is not digestible by humans, a large and ever increasing part of animal nutrition is based on highly digestive ingredients which could be used directly for human nutrition. In addition, large quantities of ruminant feed stems from forage produced in arable farming causing opportunity costs of foregone human nutrient production. While consumption patterns for milk and dairy products as well as for eggs may not be significantly affected by competition between the animal species, the differences in feed conversion efficiency have strongly influenced the share of the species in the meat market. As indicated in *Table 12*, the feed requirements for producing 1 kg meat from concentrates range from 2.9 kg in broilers to 8.6 kg in sheep. Poultry meat can, therefore, be produced much cheaper than beef or mutton. Retail prices for consumers reflect this and have led to an enormous expansion of poultry meat in relation to ruminants on a worldwide scale, while pork has maintained its position. The ongoing trend is likely to continue, because poultry meat and to a certain extent pork lend themselves easier to convenience food than ruminant meat.

Table 12.

Feed requirements for producing one kg of meat

	Feed conversion ratio, kg feed per kg lwg*	Replacement, kg feed per kg lwg*	Killing-out % of lwg*	Feed input per 1 kg of meat, kg
Broiler chicken	1.9	0.3	0.75	2.9
Pig	3.5	1.0	0.70	6.4
Cattle	5.6	—	0.70	8.0
Sheep	6.0	—	0.60	8.6

* lwg = live weight gain

Production factors: In comparison to the densely populated areas of western Europe the cost of land in Hungary, whether for purchase or for rent, should be much lower. Even when subsidy programmes will become effective following Hungary's accession to the EU and land rents may increase, land would still be much cheaper than in Western Europe.

It will also take a long time, until the cost of labour in Hungary will approach the levels of Western Europe. But the system of well capitalised family farms with highly skilled manager-labourers in Western Europe appears to be very

competitive when it comes to animai production. Despite the high cost of labour, dairy cattle and pigs still provide employment for many farming families, while poultry meat production is more concentrated and requires less labour.

The cost of capital is important in livestock farming, because of the enormous investment costs for housing and equipment, and because of the pig cycle with regularly recurring deficit phases in the production cycle. Because of the high risk and the low profit on capital that can be earned in west European farming, a high asset capitalisation is advantageous. Although interest rates for borrowed capital in Western Europe are low, they may be high in terms of real interest rates as compared to Hungary because of larger differences in the inflation rate. As inflation and interest rates are expected to decline in Hungary following entry into the EU and as the real rates of interest may rise, there may be an advantage to invest as soon as affordable.

CONCLUSION

Since the political and economic changes in 1989 Hungary's animal Industry is meeting its second great challenge within a generation: the imminent entry into the EU. With about 440 million inhabitants with reasonable incomes the EU will be the largest consumer market on earth. A favourable production environment, cheap land and labour, and a favourable geographic location in relation to large consumer markets in the EU give Hungary advantages in the market, but quota restrictions for ruminant production, highly concentrated retail markets, sensitive consumers and the prospect of greater competition from the World market raise unanswered questions. Favourable prospects are predicted for Hungary's pig and poultry industries if the necessary critical mass can be produced at competitive prices and if efficient processing and retailing channels are being established. Outside the EU the large but volatile import market in Russia lies on Hungary's doorstep.

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SUMMARY

Future strategies for animai production for the World, the EU and Hungary will have different orientations: a solution to the World food problem, meeting the demand of relatively wealthy but sensitive consumers and opening opportunities in the World market by the EU, and finding markets in the enlarged EU as well as supplying local Hungarian consumers. Good progress was made over the last 40 years to solve the World food crisis. The open question is, whether and when the fast growing economies of Asia, especially China, will start to import food of animal origin on a larger scale.

Although the EU constitutes the largest consumer market in the World, uncertainties exist regarding the competitiveness of local producers with global competitors, whose presence is to be expected from future WTO negotiations. But even the centrally administered CAP has not prevented a large disparity of producer prices in EU member states. Special production conditions imposed by society will require animal producers to be specially compensated.

Hungary has good natural conditions, a favourable geographic location and cheap land and labour to allow competitive animal agriculture within the EU. The major issues are quotas for ruminant production, market channels, critical mass of production, management skills and availability of capital. The overall prospects, especially för the pig and poultry industries, look more favourable than they have done for many years.

A JÖVŐ ÁLLATTENYÉSZTÉSI STRATÉGIÁI

MEYN, KLAUS

ÖSSZEFOGLALÁS

A világ, az EU és Magyarország állattenyésztésére vonatkozó jövőbeli stratégiák különböző irányt vehetnek. Az állattenyésztés fejlesztése, megoldást jelenthet a világ élelmezési problémájára de figyelembe kell venni a viszonylag tehetős, ámde kényesebb vásárlók igényeit is. Az EU-tagság által lehetőség nyílhat a világpiac meghódítására, a kibővült EU-ban piacra találhatnak az állati termékek, az EU-tagság révén lehetőség nyílik a magyar vásárlók támogatására is. Az elmúlt 40 év során sokat változott a világ élelmiszer-válságának megoldására irányuló törekvés. Az még nyitott kérdés, hogy vajon Ázsia gyorsan növekvő gazdaságai, főleg Kína, nagyobb mértékben fognak-e állati eredetű élelmiszert importálni, és ha igen, milyen termékeket és kitől.

Habár az EU jelenti a világpiac legnagyobb vásárlóját, bizonytalanságok még ma is észlelhetők, figyelembe véve a helyi és a nemzetközi termelők versengését, akiknek jelenléte ugyan a jövőbeli WTO tárgyalásokon kétségtelen, de még a központilag irányított CAP (Közös Agrárpolitika) sem védte ki a termelői árak nagy egyenlőtlenségét az EU tagállamaiban. A speciális termelési feltételek, melyeket a társadalom elvár (pl. környezetbarát technológiák bevezetése, az állatjólét feltételeinek biztosítása), megkövetelik, hogy az állattenyésztőket kompenzálják.

Magyarországnak jók a természeti adottságai, kedvező a földrajzi helyzete, olcsó a földje és munkaereje, ami lehetővé teszi az EU-ban való versenyképességet. A fő vitapontok a következők: a kérődzők termelésének kvótái a kereskedelmi csatornák, a termelés kritikus mennyisége, a vezetői szakértelem és az alaptőke megléte. A jövőbeli kilátások kedvezőbben alakulnak, mint sok évvel ezelőtt, főleg a sertés- és a baromfitenyésztés tekintetében.

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CLONING IN FARM ANIMALS FOR MEDICAL MODELS AND PHARMACEUTICAL APPLICATION

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Cloning techniques have been used for many decades by plant breeders. In mammals sometimes clones are naturally born as identical sibs. Microsurgically splitting of non-implanted embryos allows not to produce more than a few identical animals with the same genotype and there was no way to clone existing animals. Nuclear transfer technology includes the transfer of a donor cell (nucleus) into the cytoplasm of an enucleated metaphase II oocyte (*Wolf et al., 1998*).

Experimental cloning procedures using nucleus transfer has been tried for many years. In *Willadsen* (1986) published the first successful nuclear transplantation in sheep embryos. Afterwards cattle, rabbit, and pig embryonic cells were also used for generating cloned offspring using nuclear transfer.

Live offspring were generated in sheep after nuclear transfer of cells out of the embryonic disc, cultured *in vitro* for 6 to 13 passages (*Campbell et al., 1996*). The potential of fetal germ cells to support fully post implantation development was also demonstrated recently (*Zakhartchenko et al., 1999a*).

Pioneering experiments carried out at the Roslin Institute demonstrated for the first time that cells from adult animals can be used successfully for cloning (*Wilmut et al., 1997*). In farm animals live births have been achieved so far using somatic nuclear transfer in sheep, cows, goats, pigs and rabbits. Other species are on the way.

The successful generation of offspring in livestock animals derived after nuclear transfer depends upon a wide variety of factors like activation of the oocyte and coordination of cell cycle. Although it is now possible to produce cloned mammalian offspring from differentiated cells after transfer of nuclei, the overall success rate is currently low (only around 1% of fused karyoplast/cytoplast complexes develop till term).

Wilmut et al. (1997) proposed a so called serum starvation of donor cells as a method of choice for arresting the cells in the GO status of the cell cycle which seems to be ideal for successful reprogramming of the donor chromatin from differentiated cells. Calves have been born after nuclear transfer of serum starved fetal fibroblasts. However live calves have also been born after nuclear transfer of non-starved fetal fibroblasts (Cibelli et al., 1998; Zakhartchenko et al., 1999b).

We have evaluated the developmental potential of fetal fibroblast using nuclear transfer techniques. Nuclei of starved (8 day culture, 0,5% fetal calf serum) fetal fibroblasts from a 37-day-old fetus transferred to enucleated oocytes developed to blastocysts at a rate of 39%. Nuclei from non-starved cells showed only a development rate of 20%. Fusion rates (81%) obtained with starved fibroblasts tended to be higher than those obtained with non-starved cells (72%), cleaved at a higher rate (77% and 66%) and also the developmental capacity to blastocysts was better (39% and 20%). After transfer of blastocysts derived from non-starved and starved fibroblasts respectively 33% and 78% of recipients were pregnant on day 30. Two live calves were born from cloning non-starved fetal fibroblasts.

In a nuclear transfer program from 223 primary mammary gland cells only 63% fused and 26% developed till blastocyst stage (Zahkartchenko et al., 1999c). After transfer of 4 blastocysts to 2 recipients, 2 day 90 pregnancies were detected and 1 calf was born. This calf was a successful repetition of the sheep cloned by Wilmut et al. (1997) using mammary gland cells. Further on we were able to detect mitochondrial DNA heteroplasmy in cloned cattle produced by mammary gland cell cloning (Steinborn et al., 2000).

Completely reprogramming of nuclei from differentiated cells occurs only after nuclear envelope breakdown and chromosome decondensation. Reprogramming is initiated by a high level of maturation promoting factor (MPF) activity. Thus the activation of the oocyte should not be induced prior or soon after transfer.

Primary cultures of bovine ear skin fibroblasts were established from tissue samples of a 3-year-old cow. The tissues were cut into small pieces, treated with trypsin and transferred to culture. The skin fibroblasts did not change homozygous size and morphology till passage 10 and expressed vimentin at all passage numbers. After nuclear transfer using 92 primary ear fibroblasts 89% fused, 60% of these developed to blastocysts; and after transfer of 16 blastocysts to 12 recipients 3 day 90 pregnancies and one born calf were observed (Zahkartchenko et al., 1999c).

Somatic cumulus and oviductal cells were used as donor cells for cloning by Kato et al. (1998). Eight calves were derived from these differentiated cells demonstrating that bovine cumulus and oviductal epithelial cells of the adult have the genetic content to direct the development of newborn calves.

Adult somatic cell nuclear transfer was used to determine the totipotent potential of cultured mural granulosa cells. Nuclei were exposed to oocyte cytoplasm for prolonged periods by electrically fusing quiescent cultured cells to the enucleated metaphase II cytoplasts 4–6 h before activation. After the transfer of 100 blastocysts, survival rates on day 60, 100, 180 and term were 45%, 21%, 17% and 10% (*Wells et al.,* 1999). 10 calves were born and reared.

The generation of transgenic livestock animals using transfected cells provides numerous advantages compared to the classical methods (*Wolf et al.,* 2000):

— Integration and in some cases even expression can be evaluated *in vitro*
— No mosaics or chimeras are produced if recloning is used,
— The germ line transmission rate of hemizygot founders is 50%,
— Transformed cells can be stored frozen,
— Sex selected cells can be used,
— Time schedules are reduced,
— Costs and efforts are reduced.

Schnieke et al. (1997) demonstrated for the first time the successful transfection of ovine fetal fibroblasts and the generation of transgenic sheep by nuclear transfer. For the generation of human factor IX transgenic sheep on average 21 sheep were required for the generation of one transgenic sheep. Using DNA microinjection more than 50 sheep were required for the production of one transgenic sheep.

Actively dividing fetal fibroblasts were genetically modified with a marker gene, a clonal line was selected and the cells were fused to enucleated mature ooyctes. Out of 28 embryos transferred to 11 recipients, three transgenic calves were born (*Cibelli et al.,* 1998). Also transgenic goats were generated doing nuclear transfer techniques with transgenic fetal fibroblast.

For generation of transgenic calves we have transfected and selected fetal fibroblasts with different gene constructs. Performing more than 500 nuclear transfers the fusion rate was 85%, the developmental rate was 33% and after transfer of 95 blastocysts into 49 recipients 8 transgenic calves were born. Two of them survive today.

Gene targeting has also been achieved now by nuclear transfer from cultured somatic cells which had been gene targeted before during *in vitro* culture. *McCreath et al.* (2000) described an efficient and reproducible gene targeting in ovine fetal fibroblasts to place a therapeutic transgene at the ovine procollagene locus and the production of live sheep by nuclear transfer. The target locus was chosen in this case since the gene is highly expressed in fetal cells allowing an efficient selection process. One of the surviving lambs was hormonally induced to lactate and showed high expression of alpha 1 antitrypsin in the milk.

Kuriowa et al. (2002) prepared a human artificial chromosome vector containing the entire unrearranged sequences of the human immunoglobulin heavy and light-chain loci and introduced this vector into bovine primary fetal fibroblasts. Selected cells were used to produce cloned fetuses and healthy transchromosomic calves in which human immunoglobulin proteins could be detected.

One very important application of nuclear transfer of transfected cells is the genetic modification of pigs for xenotransplantation. Natural antibodies in plas-

ma of human beings bind specific carbohydrate residues on the surface of pig cells. Thus pig organs transplanted to human beings are destroyed by hyperacute rejection within a few minutes. Inactivation of the enzyme alpha 1,3 galactosyltransferase should help to overcome this problem. Several groups have published successful generation of cloned transgenic pigs with homologous recombination in this target locus.

As shown cloning farm animals using modified cultured cells provides more efficient generation of genetically defined new medical models and pharmaceutical applications.

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SUMMARY

Experimental cloning procedures using nucleus transfer has been tried for many years, and success has been documented in sheep, cattle, rabbits, goats and pigs. Successful cloning of animals has been achieved by transferring the nucleus from differentiated cells including mammary tissue, ear skin fibroblasts, and somatic cumulus and oviductal cells as well as from fetal fibroblasts to the cytoplasm of an enucleated oocyte. Various techniques to enhance success of nuclear transfer methodology are discussed as well as the numerous advantages of using transfected cells to generate transgenic livestock for new medical and pharmaceutical applications.

GAZDASÁGI ÁLLATOK KLÓNOZÁSA ORVOSI ÉS GYÓGYSZERTERMELÉSI CÉLRA

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ÖSSZEFOGLALÁS

Már évek óta folytatnak sejtmag átültetéses kísérleti klónozási műveleteket, és sikereket értek el juhoknál, marháknál, nyulaknál, kecskéknél és sertéseknél. Oly módon hajtották ezt végre, hogy elkülönített sejtekből, pl. emlő szövetekből, fül bőr fibroblast-ból, szomatikus kumulusz-ból, méhkürt sejtekből vagy magzati fibroblast-ból, a sejtmagot átültették egy sejtmagtalanított enuckleált oocyta (az érett petesejt fiatal alakja) citoplazmájába. Az előadásban megvitatásra kerülnek a sejtmag átültetés módszertanának sikerét szolgáló különböző technológiák és az átültetett sejtek használatának számos előnye a génátültetéssel előállított haszonállatok új orvosi és gyógyszerészeti alkalmazásának aspektusai.

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STEM CELL TECHNOLOGIES IN CATTLE AND HUMAN

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Methods for derivation of embryonic stem (ES) cells *in vitro* are well established in some mammalian species. ES cells are undifferentiated cells derived from the preimplantation stage of embryos, which can give rise to any cell type. Establishment of ES cells has been reported for mouse(*Evans and Kaufman,* 1981; *Martin,* 1981), hamster (*Doetschma, et al.,* 1988), mink (*Sukoyan et al.,* 1993), pig (*Wheeler,* 1994), rhesus monkey (*Thomson et al.,* 1995), common marmoset (*Thomson et al.,* 1996), chicken (*Pain et al.,* 1996), bovine (*Sims and First,* 1994; *Cibelli et al.,* 1998 *Mitalipova et al.,* 2001) and human (*Thomson et al.,* 1998; *Reubinoff et al.,* 2000). The essential characteristics of all ES cells are prolonged proliferation *in vitro* and the ability to form differentiated cell types. One of the most important achievements in mammalian embryology was the targeted mutation of mouse ES cells by homologous recombination. Similar applications are of interest for livestock species. Despite tremendous research effort to establish ES cells for livestock, there is no evidence of germ line transmission of ES cells in any species other than in the mouse.

There are at least several pluripotent embryonic cell types: ES cells, embryonic carcinoma (EC) cells and embryonic germ (EG) cells. In mice, pluripotent embryonic cells were first derived from teratocarcinomas that contained an undifferentiated cell population termed embryonic carcinoma cell lines (*Evans and Kaufman,* 1981; *Martin,* 1981). EC cells differentiate into all three germ layers *in vitro* but have several disadvantages. For example, the EC cell lines have karyotypic abnormalities and low germ line transmission.

Another population of pluripotent stem cells was isolated from germ cells and termed embryonic germ (EG) cells. In this case, primordial germ cells migrating from the gonadal ridge are isolated and maintained on feeders. Also like ES and EC cells, EG cells differentiate into derivatives of all three embryonic germ layers, but remain euploid.

A third population, embryonic (ES) stem cells, are isolated generally from the ICM of blastocysts and continuously passaged on irradiated feeder layers (*Evans and Kaufman,* 1981; *Martin*, 1981)

Initial attempts to culture bovine ICM cells and presumed ES cells using mouse ES cell technology, resulted in long term *in vitro* culture and demonstrated pluripotency, but not totipotency (*Keefer et al.,* 1994; *Stice et al.,* 1996) as tested by chimerism or nuclear transfer (NT). Nuclear transfer offspring have been produced from the following cell types: 1) bovine ICM cells cultured in microdrops for 4 weeks, with approximately four microdrop passages (*Sims and First,* 1994), 2) sheep embryonic disc cells cultured for up to 13 passages and (*Campbell et al.,* 1996; *Wells et al.,* 1997), 3) primordial germ cells of pigs (*Piedrahita et al.,* 1998). Using chimera formation as a method to determine pluripotency, transgenic chimeric pigs were obtained from cultured embryonic cells (*Piedrahita et al.,* 1998), and chimeric calves were produced from long-term (ten passages) bovine embryonic stem cells (*Cibelli et al.,* 1998). Also, bovine ES cells were isolated from precompacting 8–16 cell stage embryos and had proliferated for over 3 years and showed pluripotency *in vitro* (*Mitalipova et al.,* 2001).

ES cells are totipotent cells, capable of differentiating into the precursor cells for any cell type, including functional germ cells. For animal reproduction and genetic manipulation, these cells must also be totipotent after extensive culture *in vitro*, and this may require immortalization of the cell line. The reason why ICM — or 8–16 cell stage — derived ES cells are totipotent after short-term passage is probably due to increased chromosomal abnormalities with advanced passages (*Mitalipova et al.,* 2001). It is not surprising that immortal cells become aneuploid and polyploid. There is evidence in mice that totipotency of ES cells can be reduced after extensive culture *in vitro* or regained by passaged primordial germ cells for a methylation-inactive gene (*Stewart et al.,* 1994). It has been suggested that one change that could affect totipotency of cultured cells is a progressive demethylation of genes of cultured cells to reduce totipotency or a post-mature demethylation, resulting in expression of a methylation-inactivated gene, such as Xist (*Stewart et al.,* 1994). Based primarily on mouse studies (*Brandeis et al.,* 1993; *Surani et al.,* 1993) both sperm and egg enter fertilization with DNA moderately methylated, also sperm are hypomethylated compare to the oocyte. The DNA of cleavage-stage embryos is poorly methyllated but becomes extensively methylated at late blastocyst stage, the time when ES cells are derived from ICM cells (*Surani,* 1993); (*Brandeis et al.,* 1993). Long-term passaged cell lines suffer from chromatin damage and deletion, most often due to telomere shortening associated with the extended culture period. This problem is avoided by immortalizing the cell line (*Bodnar et al.,* 1998; *Counter* 1996). Theoretically the precursor cells of the totipotent ICM cells should also be totipotent, unless their genome has not acquired a mature methylation pattern sufficient to allow totipotency.

We have derived bovine pluripotent cells from 8–16 cell stage of embryos which have been cultured and passaged *in vitro* for more than 150 passages (*Mitalipova, et al.,* 2001). Morphologically, these cells resembled ES cells of other species, indefinitely proliferated *in vitro*, and remain potential to differentiate into derivatives of all three germ layers. Mouse, human, and primate ES cells grow as multilayer colonies with a distinctive margins and high nuclear-cytoplasmic ratios (*Evans and Kaufman,* 1981; *Thomson et al.,* 1995; *Thomson et al.,* 1998; *Reubinoff et al.,* 2000). Bovine embryonic cells also have a high nuclear-cytoplasmic ratio, but grow as monolayer colonies with a high density of lipid inclusions. They express all three stage specific embryonic antigens SSEA-1, -3 and -4 and c-Kit receptor. Long-term culture of bovine cell lines resulted in abnormality of karyotype. Also, mouse ES cells cultured for more than 20 passages *in vitro* become aneuploid and the cell lines with more than 50% abnormal karyotype are never able to contribute to the germ line of aduit chimera (*Longo et al.,* 1997).

Clonal derivation of bovine embryonic cells is complicated by the difficulties associated with dissociation into single cells. Unlike mouse, primate or human ES cells, bovine cells are more sensitive to any enzymatic disaggregation. Alternative clonal methods need to be developed. This will be important in the use of single ES cell for gene targeting experiments in making transgenic animals.

Cibelli et al. (1998) established bovine ES-like cells that proliferated for more than 12 months without differentiation and displayed ES cell characteristics. In one experiment, blastocyst outgrowth was cultured and embryo-derived cells were transfected by DNA microinjection of a ß-galactosidase (ß-Gal)-neomyocin (ß-geo) expression vector. In another experiment, bovine fetal fibroblasts were transfected with the same vector and used as a donor nuclei in NT experiments to produce transgenic NT embryos. These blastocyst stage NT embryos were used to derive ES-like cells. Morula injection of both the embryo- or nuclear transfer-derived transgenic ES-like cells resulted in the birth of nine chimeric calves carrying the ß-Gal transgene in at least one of the tissues investigated. These were the first transgenic calves obtained by use of long-term cultured pluripotent embryonic cells.

Several attempts to derive bovine pluripotent cell lines from PGCs were successful. Genital ridges were obtained from fetuses 29 to 70 days post conception (*Cherny et al.,* 1994; *Lavoir et al.,* 1994; *Forsberg et al.,* 2002). The PGCs were isolated either by enzymatic or mechanical dissagregation and cultured on embryonic mouse fibroblasts. Pluripotency was evaluated by formation of embryoid bodies (*Cherny et al.,* 1994) and by injection into blastocyst, demonstrating that FITC-labelled PGCs integrated into ICM of a chimeric embryo. Nuclear transfer of cultured PGCs resulted in a live birth (*Forsberg et al.,* 2002; *Pace et al.,* 2002).

Human ES cells

Human ES cells have been derived in many laboratories (*Thomson et al.,* 1998; *Amit et al.,* 2000; *Reubinoff et al.,* 2000; *Amit et al.,* 2002; *Richards et al.,* 2002;); http;//escr.nih.gov; *Mitalipova et al.,* 2003 submitted). All human ES cell lines were isolated from the ICM of *in vitro* fertilized embryos at the blastocyst

stage. We have derived four human ES cell lines from poor quality embryos (*Mitalipova et al.,* 2003 submitted). The initial isolation of human ES cells, like bovine ES-like cells, used mechanical dissagregation of ICM cells. Further maintenance of these cells utilized different dissagregation agents. Unlike bovine ES cells, one of the human ES cell lines, H9, was capable of undergoing clonal propagation. Two subclones, H9.1 and H9.2 were isolated from single cells, which expressed the markers as parent H9 cell line and generated teratomas (*Amit et al.,* 2000). Also, it has been reported that other human ES cell lines, H1, H13, I3 and I6 have been assessed for single cell cloning (*Amit et al.,* 2002).

One of the remarkable characteristics of human ES cells, compared to bovine or murine ES cells, is their capability to maintain stable karyotype during prolonged *in vitro* culture. We and other groups have shown that human ES cells cultured for more than a year retain a normal karyotype. Male and female hES cell lines that maintain a normal karyotype after long term passaging, have been described by several laboratories (*Thomson et al.,* 1998; *Amit et al.,* 2000; *Reubinoff et al.,* 2000; *Xu et al.,* 2001). In contrast, most female mouse ES cell lines and bovine ES-like cells have a tendency to loose one of the X chromosome, as was mentioned above.

The panel of cell surface markers used to characterize human ES cells are similar to those used for mouse and bovine ES cells. Additionally, those were used to characterize human EC cells. Similar to mouse ES cells, the hES cells express alkaline phosphatase (*Thomson et al.,* 1998; *Reubinoff et al.,* 2000; *Xu et al.,* 2001; *Amit et al.,* 2002; *Mitalipova et al.,* 2003) submitted to Stem Cells). In addition, the undifferentiated hES cells express the globoseries of glycolipid antigens SSEA-3 and SSEA-4, which are also expressed by human EC cells and bovine ES-like cells. In contrast to the mouse ES cells and similar to bovine ES-like cells, hES cells lack expression of a SSEA-1 antigen. In addition, the tumor-recognition antigens, TRA-1-60 and TRA-1-81 (*Henderson et al.,* 2002) expressed by hES cells. The transcription factors have also been used to characterize hES cells. The POU transcription factor, Oct-3/4, is expressed in hES cells and down-regulated upon differentiation (*Lebkowski et al.,* 2001; *Xu et al.,* 2001). We have demonstrated that hES cells maintained for over 1 year in continuous culture retain Oct-4, SSEA-3, -4, TRA-1-60 and TRA-1-81 expression (*Mitalipova et al.,* submitted 2003). It is still unclear at this time if any, of these markers will be the most sensitive to characterize the undifferentiated status of human or bovine ES cells. This indicates that more fundamental studies need to be performed to fully characterize not only human but also ES cells from domestic species.

One of the definitive characteristic of ES cells is the ability to differentiate into derivatives of all three germ layers. The standard way to assess this capacity is the formation of teratomas. Normally, hES cells are injected into immuno-compromised mice and three to four months later, teratomas form and contain endoderm, mesoderm and ectoderm cell types.

Human ES cells have been successfully differentiated into cells of many lineages. Typically, ES cells are differentiated by aggregation of cells into structures called "embryoid bodies" (EBs). These EBs usually consist of cells from all three germ layers that, under influence of different growth factors, differentiate

into more complex mature phenotypes. Although, most differentiation protocols are based on formation of EBs, it is also possible to differentiate human ES cells directly into specific cell phenotypes without EB formation (*Mitalipova et al.*, unpublished data).

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SUMMARY

Originally, ES cells have been used for the study of early mammalian embryogenesis, e.g. stage-specific gene expression. ES cell-mediated transgenesis has advantages over other methods of making transgenic animals: integration of viruses into early embryos or microinjection of DNA into the pronucleus of freshly fertilized oocyte which associated with random integration of DNA (*Chan, et al.* 1998). ES cell technology increased the efficiency of producing transgenic animals, since individual cell clones derived from a single transfected cell can be screened *in vitro* for the integration and expression of the exogenous DNA construct before creating a transgenic chimeric animal. Since ES cells are immortal cell lines and proliferate rapidly, they facilitate homologous recombination (*Capecchi,* 1989). This technique allows the precise modification of existing genes, overcomes positional effects and insertional inactivation and mediates the inactivation of specific endogenous genes (*Osterrieder and Wolf,* 1998). Recent progress in nuclear transfer using transfected cells offers an alternative method for producing transgenic animals, especially in species where ES cells are difficult to isolate. But the efficiency of the NT procedure and the normal development of the clones remains to be improved. The advantage of using ES cells for NT in the mouse is that ES cell-derived clones have higher survival rate to term (*Rideout et al.,* 2000). Homologous recombination requires the selection of transfected cells and propagation of these cells to identify the correct targeted genes. This strategy can be limited by low number of cell divisions (*Cibelli et al.,* 1998). Therefore, the establishment of pluripotent cells is still an elusive goal in farm species.

Much attention has been devoted recently to potential application of human ES cells in biology and medicine encompassing: basic embryological research, functional genomics, growth factors and drug discovery and cell therapy. Directed differentiation of hES cells using growth factors could enable the selection of pure, committed progenitor cells from spontaneously differentiated cells.

HUMÁN ÉS SZARVASMARHA ŐSSEJT-TECHNOLÓGIÁK

MITALIPOVA, MAYA — STICE, STEVE

ÖSSZEFOGLALÁS

Az őssejteket eredetileg az emlősök embriogenezisének tanulmányozására használták. Az őssejt által közvetített transzgenezis előnyösebb más transzgénikus állat előállítási módszereknél, mint pl. a korai embrióba vírus vektorral történő génbevitelnél, vagy a mikroinjektálás útján való génátültetésnél. Az őssejt-technológia növeli a transzgénikus állat előállítás hatékonyságát, mivel az egyetlen tisztított sejtből származó sejtrendekben jól nyomon lehet követni a bevitt DNS integrációját mielőtt létrehoznák a transzgénikus kimérát. Mivel az őssejtek halhatatlan sejtvonalak és gyorsan szaporodnak, elősegítik a homológ rekombinációt. A módszer javítja a natív gének pontos modifikációját, kiküszöböli a pozicionális hatásokat és az inzercióból származó esetleges inaktivációt. A tisztított őssejtek felhasználásával történő sejtmagértékelés újabb lehetőséget kínál a transzgénikus állatok előállítására, főleg olyan fajokban, ahol az őssejteket nehéz izolálni. A sejtmag-átültetés hatékonysága és az előállított klónok normális fejlődésének biztosítása még további fejlesztésre váró technika. Az egerekben például, az őssejtek előnye a sejtmag-átültetéses módszerrel szemben az, hogy az őssejtekből előállított klónoknak nagyobb esélyük van a túlélésre. A homológ rekombinációhoz szükséges a tisztított sejtek szelekciója és ezek elszaporítása fontos mozzanat azért is, hogy meg lehessen határozni a helyes célgéneket. Ezt a stratégiát korlátozni lehet kevés számú sejtosztódással. A pluripotens sejtek létrehozása azért még mindig nehezen elérhető célnak számít a gazdasági állatok esetében.

Napjainkban a humán őssejtek felhasználásának lehetőségeit intenzíven vizsgálják, mert ezek felhasználása széles körű lehetőséget kínál az embriológia kutatások, a humán orvosi vizsgálatok és a gyógyítás, valamint a biológia más területein (növekedési faktorok kutatása gyógyszer-előállítás, sejtterápia).

HADLACZKY, GYULA

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ARTIFICIAL CHROMOSOMES IN ANIMAL PRODUCTION

HADLACZKY, GYULA

INTRODUCTION

Although, the term biotechnology was introduced only 86 years ago by the Hungarian mechanical engineer Ereky, Károly (later Minister of Agriculture), the first biotechnology "applications" such as domestication of animals go back to the prehistoric times. In the 20th century, the introduction of recombinant DNA technology established the fundamentals of modern biotechnology that offers incredible potential for the future, and probably for the survival of mankind. Conventional agricultural technologies can not meet the current demand for food of the existing human population, and can not keep pace with the increasing need for food, in the 21st century. At the moment, biotechnology appears to be the only feasible and acceptable strategy for our species in this "struggle for life".

In the animal biotechnology, the genetic modification of animals by the introduction of new genetic traits is one of the prime approaches to increase the production efficiency. In most cases of such genetic engineering, vectors that carry the payload DNA are necessary not only for the transfer of the genetic information but also for the proper function of them in the recipient cells, tissues or organisms. For those applications where the genetic modifications require

the introduction of large or multiple genes, and their regulated and stable expression is desired, mammalian artificial chromosome vectors may be an attractive tool.

In the last decades, considerable efforts were made to develop stable mammalian artificial chromosomes. Among the different approaches, satellite DNA-based artificial chromosomes (SATACs) represent the most advanced artificial chromosome technology (for a review see *Sumner*, 2002).

In this paper, those milestones of the development of SATAC technology are summarized that established the feasibility of the use of the artificial chromosomes in biotechnology.

Generation of satellite DNA-based artificial chromosomes

In vivo generation of satellite DNA-based artificial chromosomes is an induced amplification-dependent *de novo* chromosome formation process, in cultured mammalian cells. This includes:
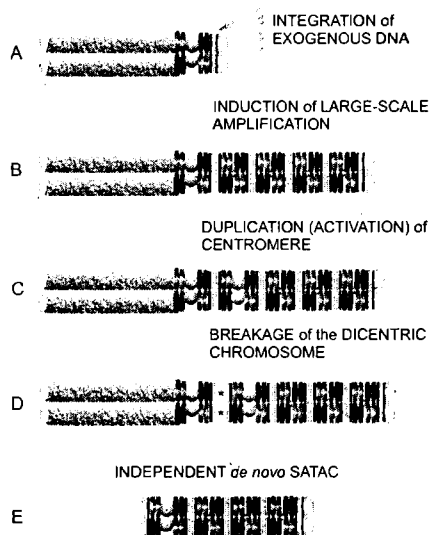
— Targeted integration of "foreign" DNA (selectable marker, useful "payload" DNA) into the pericentromeric satellite DNA region of certain chromosomes of the host cell (*Fig. 1a*),

— Integration initiates large-scale amplification of the integration site that lead to the formation of *de novo* chromosome arm (*Fig. 1b*),

— Activation of amplified centromeric region results in the formation of dicentric chromosome (*Fig.1c*),

— Breakage of dicentric chromosome separates the amplified chromosome arm from the host chromosome, which results in an independent *de novo* chromosome (SATAC) composed of co-amplified satellite, telomeric, and "foreign" DNA sequences (*Fig. 1d,e*).

Fig. 1.: **Subsequent steps of the generation of satellite DNA-based artificial chromosomes**

A   INTEGRATION of
           EXOGENOUS DNA

           INDUCTION of LARGE-SCALE
           AMPLIFICATION

B

           DUPLICATION (ACTIVATION) of
           CENTROMERE

C

           BREAKAGE of the DICENTRIC
           CHROMOSOME

D

           INDEPENDENT *de novo* SATAC

E

These newly formed chromosomes are built up by one or more inverted units (amplicons), and the bulk of their DNA are satellite sequences characteristic to the chromosome region of the integration site. Except the foreign DNA these *de novo* formed chromosomes acquire all the structural and functional elements (centromere, telomere, origin of replication) from the endogenous sequences of the host chromosome. Therefore, the formation of a stable *de novo* chromosome, at the same time, is the ultimate test of the functionality of these components together with the integrated exogenous genetic material. SATACs are heterochromatic, however, the presence of a dominant selectable marker gene on these chromosomes ensures that SATACs provide suitable chromatin environment for high level gene expression (for more details see *Kereső et al.,* 1996; *Holló et al.,* 1996; *Csonka et al.,* 2000)..

SATACs can be generated in cells of different mammalian species, and different mouse, hamster, and human SATACs have already been generated (*Fig. 2ac*). The carrying capacity of SATACs is unlimited, artificial chromosomes with large payload (>1 million base pairs) have been produced (*Fig. 2d*). Considering the similarities of cellular and chromosomal characteristics of higher eukaryotes, the methodology for generation of satellite DNA-based artificial chromosomes may well be applied in wide rage of species.

Purification of SATACs

Because of the amplified satellite DNA sequences, the DNA composition (A/T:G/C ratio) of SATACs differs significantly from that of the "natural" chromosomes. This allows the purification of SATACs by flow cytometry. Using a dual laser-beam fluorescence cell sorter (FACS), SATACs can be routinely sorted at rates greater than 1 million per hour (deJong et al, 1999). Depending on the size and DNA base pair composition of the SATACs, the purity of separated artificial chromosomes is >96–99% (*Fig. 3*). Chromosome transfer and transgenic animal experiments (see later) proved that flow cytometry is sufficiently mild to provide structurally and functionally intact purified artificial chromosomes. Purification of artificial chromosomes makes possible the large-scale, industrial use of them, and purified SATACs can be subjected to those quality control analyses that will be necessary to meet the safety requirements.

Delivery of SATACs

A prerequisite for the use of artificial chromosomes in different applications of biotechnology is their efficient transfer into recipient cells. "Low efficiency" delivery methods like cell fusion, or microcell-mediated chromosome transfer were sufficient to prove that SATACs can function and were stably maintained in cells of different species (mouse, hamster, bovine and human (*Telenius et al.,* 1999).

Large-scale purification of artificial chromosomes opened the way for development of efficient delivery methods such as cationic lipid and cationic dendrimer- mediated transfer (*deJong et al.,* 2001) or to develop new microinjection techniques for the direct transfer of artificial chromosomes into oocytes (*Co et al.,* 2000; *Wang et al.,* 2001; *Monteith et al.,* 2003).

Fig. 2.: **SATACs of different mammalian species**
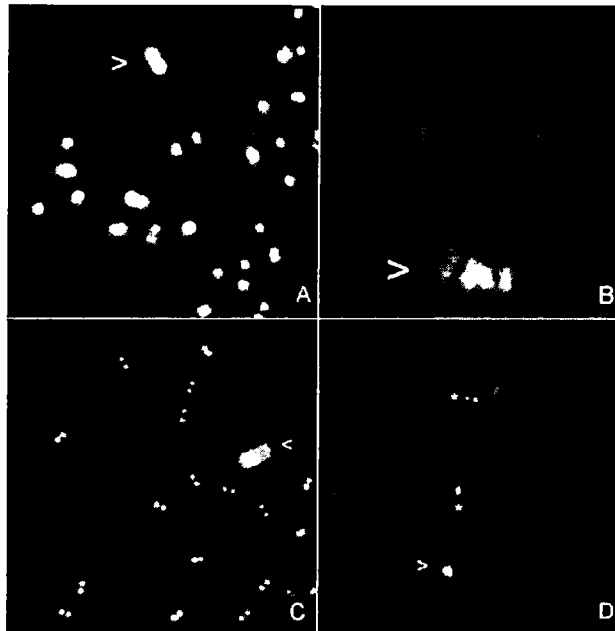
A: Mouse SATAC (arrowhead). *In situ* hybridization with mouse major satellite sequences (yellow signals) that specific to the pericentromeric regions of mouse chromosomes demonstrates that the main DNA component of the SATAC is satellite DNA. This SATAC was used to generate the first transgenic anima with artificial chromosome (see later) Chromosomes are counterstained with propidium iodide (red).
B: Hamster SATAC (arrowhead). Gree signals correspond to satellite sequences, and red signals show the presence of "foreign" DNA. Chromosomes are counterstained with DAPI (blue).
C: Human SATAC (arrowhead). Double green dots on the chromosomes correspond to the centromeres, red and green signals on the SATAC show different satellite DNA sequences. Chromosomes are counterstained with DAPI (blue).
D: Human SATAC (arrowhead) with large payload. This tiny artificial chromosome carries >1.5 million base pairs of a payload genomic sequence (green signal) with lox sequences for homologous recombination (see later). For a comparison see a 120 kilobase pairs stretches of the same sequences on natural chromosomes (asterisks). Red signals correspond to satellite DNA sequences. Chromosomes are counterstained with DAPI (blue).

Transgenic animals with artificial chromosome

An important milestone in validating the artificial chromosome technology was the successful generation of transgenic mouse with purified SATACs (Co et al., 2000). It has proved that phenotypically normal, fertile animals can be produced with inserting an artificial chromosome into their genome. The additional artificial chromosome (Fig. 4a) was stably maintained and passed through at least four generations without any adverse effect. In respect to the biotechnology applications, it is equally important that transgenic mouse has also been generated with an artificial chromosome that carried a mammary gland specific expression system (Fig. 4b). This mouse secreted a therapeutic protein, in her milk, at each of the three lactation cycles (unpublished).

Fig. 3.: **Separation of SATACs with FACS. Because of the unique DNA content of the SATACs they differ significantly from natural chromosomes, and can be separated efficiently**
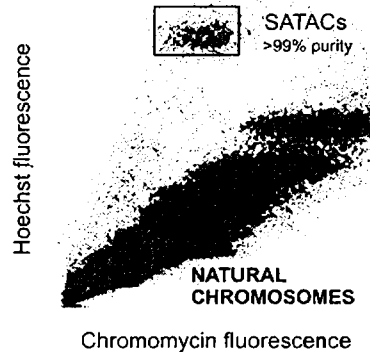
On this flow, karyotype each dot corresponds to a single chromosome

From these results we can conclude that:

— With appropriate artificial chromosome the species-specific chromosome number can be changed without any detrimental effect;

— The artificial chromosome can be inherited through generations;

— Persistent tissue specific gene expression can be achieved from artificial chromosomes.

Engineering of SATACs – platform ACE

In the basic process of generation of SATACs, each different payload DNA requires the construction of a new artificial chromosome.

Fig. 4.: **Transgenic animals with SATACs**
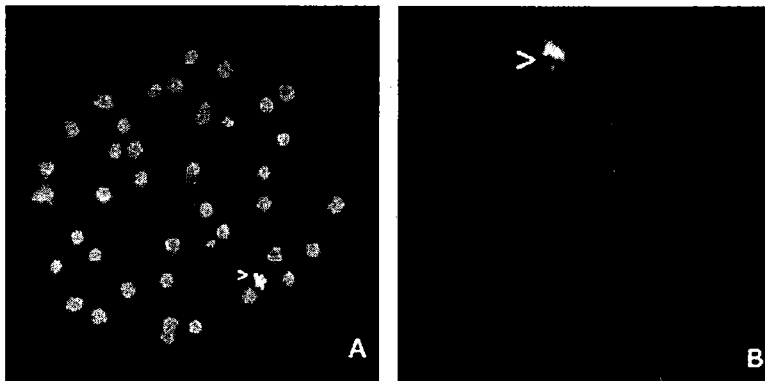
A: SATAC (arrowhead) in the lymphocyte of the first transgenic mouse (2n=41). Green signals show the "foreign" DNA sequences on the SATAC, red signals correspond to mouse major satellite sequences. Chromosomes are counterstained with DAPI (blue).
B: Chromosomes of a transgenic mouse (2n=41) with a SATAC (arrowhead) that is providing tissue–specific expression of the transgene. Green signals correspond to the payload sequences, chromosomes are counterstained with DAPI (blue).

In order to increase the efficiency of chromosome engineering, homologous recombination system (cre/lox) can be inserted into an existing artificial of chromosome (*Stewart et al.,* 2002), or artificial chromosome can be generated with exogenous DNA that contains the lox sequences necessary for the recombination (*Fig. 1d*). Although, this system doesn't allow successive loading of payload DNA, appropriate gene construct can be inserted onto such artificial chromosome in a single engineering step.

Alternatively, artificial chromosome can be generated with "foreign DNA" that contain sequences sufficient for unidirectional homologous recombination. Such satellite DNA-based artificial chromosome has successfully been constructed with a integrase-mediated site-specific recombination system (unpublished). This artificial chromosome with multiple acceptor sites acts as a blank cartridge onto which the gene(s) can be inserted without having to re-engineer the chromosome. This new generation of SATAC and its supporting components are referred to as the ACE (Artificial Chromosome Expression) system. The ACE system allows the rapid and efficient construction of "custom" artificial chromosomes with any cloned genes, in a single or multiple successive loading of useful gene(s), or gene complexes with specific controlling elements.

CONCLUSIONS

In recent years, from the basic science level satellite DNA-based artificial chromosomes have been developed to a non-integrative vector system. These "ready-to-use" artificial chromosome vectors with a large DNA-carrying capacity appear to be a very promising tool in different fields of biotechnology. In the near future, they can be a valuable addition to the trangenics technology for producing higher quality nutrients, or bioactive molecules and therapeutic proteins of value in human and veterinary medicine. By the steady increase of the number of the available cloned genes that confer to disease resistance, growth and reproduction control, tolerance to environmental or "technological" stresses, artificial chromosome vectors may play a prime role in the complex genetic modification of animals.

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SUMMARY

In vivo generation of mammalian artificial chromosomes represents a reproducible and efficient technology for construction of stable satellite DNA-based artificial chromosomes (SATACs) with defined genetic content.

SATAC technology is based on the induction of de novo chromosome formations via large-scale amplification, which can be initiated by targeted integration of exogenous DNA into the satellite/rDNA region of host chromosomes. Co-amplification of sequences of the integration site results in de novo formed chromosome arms and new chromosomes that composed of exogenous DNA and satellite/rDNA sequences. SATACs are heterochromatic, however, they provide a suitable chromosomal environment for stable, persisting expression of the integrated exogenous genetic material.

SATACs can be engineered, purified and transferred into recipient cells including fertilized eggs. Transgenic animals have successfully been generated with purified SATACs, and the transmission of artificial chromosome through generations has been demonstrated.

Due to the rapid development of SATAC technology, the feasibility of the use of satellite DNA-based artificial chromosome has been established in different fields of biotechnology. SATACs represent a novel protein production platform both for cellular protein production and for production of therapeutic molecules in body fluids of transgenic animals. Also, stable and heritable SATACs with practically unlimited carrying capacity may serve as potential vectors for animal breeding.

MESTERSÉGES KROMOSZÓMÁK AZ ÁLLATTENYÉSZTÉSBEN

HADLACZKY, GYULA

ÖSSZEFOGLALÁS

A mesterséges kromoszómák *in vivo* generációja hatékony segítséget jelent a meghatározott genetikai tartalommal rendelkező stabil szatellit DNS-alapú mesterséges kromoszómák (SATAC-ok) előállításához.

A SATAC technológia a *de novo* kromoszóma formáció nagymértékű amplifikációs indukcióján alapul, ami előidézhető exogén DNS-nek a befogadó kromoszóma szatellit/DNS régiójába való célzott integrációjával.

A szekvenciáknak a bejuttatás helyén való egyidejű amplifikációja olyan *de novo* kromoszóma-ágak és új kromoszómák kialakulásához vezet, amelyek a külső DNS és a szatellit/rDNS szekvenciáiból épülnek fel. A SATAC-ok heterokromatikusak, azonban megfelelő kromoszóma környezetet biztosítanak az integrált exogén génanyag stabil, tartós kialakulásához.

A SATAC-okat ki lehet alakítani, purifikálni majd bejuttatni a befogadó sejtekbe, többek között megtermékenyített petesejtekbe is. Sikerült transzgenikus állatokat létrehozni purifikált SATAC-ok segítségével, és a mesterséges kromoszóma több generáción át való öröklődését kimutatni.

A SATAC technológia gyors fejlődése következtében a biotechnológia számos területén van lehetőség a szatellit DNS-alapú mesterséges kromoszómák alkalmazására. A SATAC-ok új protein előállítási platformot jelentenek a sejtprotein előállításához és a transzgenikus állatok testfolyadékainak terapeutikus molekuláinak létrehozásához. A stabil és öröklődni képes, gyakorlatilag határtalan teherbírással rendelkező SATAC-ok potenciális vektorokként szolgálhatnak az állattenyésztés számára.

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BIOTECHNOLOGY IN ANIMAL BREEDING

ZINOVIEVA, NATALIA — BREM, GOTTFRIED

Transgenics is one of the fastest growing areas of animal breeding research. Since 1981, when the term 'transgenic' was first used (*Gordon and Ruddle,* 1981), there has been rapid development for applications of genetically engineered animals in the different areas of both science and praxis. For example, in Great Britain between 1990 and 1999, the number of transgenic animals increased more than ten-fold (from 48,255 to 511,607).

The principal strategies for genetic modifications of farm animals involve germline and somatic gene transfer approaches. Germline transgenic animals carry a transgene in all tissues including the gametes. In contrast, using somatic gene transfer the genetic information of the germline is not involved, i.e. the foreign DNA is not transmitted to progeny.

The first germline transgenic farm animals were created nearly 20 years ago by microinjection of foreign DNA into the pronuclei of the zygotes (*Brem et al.,* 1985, *Hammer et al.,* 1985). Later numerous different techniques were developed for this purpose (*Pinkert and Murray,* 1999).

Current applications of germline gene transfer in farm animals include the improvement of product quality and quantity, disease resistance, the production of valuable proteins in the mammary gland or other bodily fluids, for example blood or urine, the genetic modification of tissues and organs for xenotransplantation and the generation of new animal models (*Müller and Brem,* 1998; *Wolf et al.,* 2000). Somatic gene transfer is mainly used for gene therapy and bioproduction.

One of the most successful applications of transgenesis is the genetic modification of farm animals for the production of heterologous proteins of high value ("gene farming") (reviewed by *Lubon,* 1998). The mammary gland is the organ of choice for this application. When analyzing the cost for protein produc-

tion using different methods, transgenic technology is 2–3 times cheaper than fermentation or cell culture facilities (*Hodgson,* 1992).

To obtain mammary gland specific expression, regulatory sequences of the milk protein genes encoding caseins, α-lactalbumin, β-lactoglobulin or whey acidic protein should be included into the gene constructs (*Wilmut et al.,* 1990; *Brem et al.,* 1993; *Maga and Murray,* 1995).

Generations of transgenic rabbits, pigs, goats, sheep and cattle expressed recombinant proteins in concentrations up to 35 g/l milk and also stably transmitted the transgenes to their offspring were reported (*Janne et al.,* 1998).

One of the main criterions for choosing the species for gene farming is the quantity of protein needed per year. Most proteins are required in the range of kilograms per year. Therefore, rabbits appear to be the most suitable species for this aim (*Castro et al.,* 1999). Also, the efficiency of the germline gene transfer in rabbits is relatively high. Rabbits produce at least 4 litres milk per lactation period (*Brem et al.,* 1994) and can be milked semi-automatically. In addition, rabbits can be kept under specific pathogen free conditions (*Cere et al.,* 1997).

Mammary gland specific expression of recombinant proteins in rabbits has been achieved using WAP, α-lactalbumin, κ-casein, β-lactoglobulin and α_{S1}-casein-promoter driven gene constructs (*Brem et al.,* 1994, 1995; *Zinovieva et al.,* 1998; *Castro et al.,* 1999; *Coulibaly et al.,* 1999, 2002; *Hiripi et al.,* 2003). The expression levels of these recombinant proteins in rabbit milk were different and ranged from 50 ng/ml for human interleukin-2 (*Bühler et al.,* 1990) to 10 mg/ml for bovine chymosin (*Brem et al.,* 1995).

The use of transgenic rabbits for the production of recombinant proteins in milk is relatively time efficient due to a short generation interval. Using large farm animals (sheep, goats, and cows) for this aim has the disadvantage of a long time from the start of the experiments to the expression of recombinant proteins in the milk. To target the expression of a recombinant gene construct into the milk of large farm animals the methods of the somatic gene transfer are discussed. Although some improvements have been reported, naked DNA induces transient expression only in a limited number of cells. Several approaches are currently being used to achieve local gene delivery into the mammary gland of adult animals: particle bombardment, electroporation, virus-based vectors (retroviruses and adenoviruses), liposomal transfer and receptor-mediated gene transfer (*Zelenin et al.,* 1991; *Sobolev et al.,* 1998; *Dühler et al.,* 2002). The main disadvantages of retrovirus-mediated gene transfer are the restriction in the size of the DNA-construct that can be introduced into the vector (several kb), the capacity to infect only dividing cells and the host immune response (*Rollins et al.,* 1996, *Ghazizadeh et al.,* 1997). Adenoviral vectors are highly efficient and can be used to transduce non-dividing cells also; however they are not integrated into the host genome which results in a very short expression period. The liposomal approach has disadvantages associated with the cytotoxity of Lipofectin and the *in vivo* instability of liposomes. The attractive feature of receptor-mediated gene delivery is that it provides an opportunity to achieve cell specific delivery of DNA complexes, whereas the disadvantages are associated with the variability in binding capacity of the conjugate and with the difficulty to obtain receptor ligands of high purity in sufficient quantities.

The technique of the direct infection of the mammary gland using a retrovirus gene construct was first applied by Archer to produce the human growth hormone in the mammary gland of goats in concentrations up to 60 ng/ml (*Archer et al.,* 1994). Using the method of direct multiple injections of the retrovirus gene constructs into the mammary gland of pregnant animals we could achieve the expression of recombinant erythropoetin in pigs, goats and cattle up to 1 mg per litre milk (*Zinovieva et al.,* unpublished). Combining the hyperosmotic pretreatment and receptor-mediated gene transfer technology, *Düchler et al.* (2002) achieved expression levels of recombinant proteins in sheep milk of more than 1 µg/ml. *Sobolev et al.* (1998) used the method of receptor-mediated endocytosis and estimated that the production of foreign proteins in sheep milk was more than 0,6 ng/ml. The main interest of the somatic gene transfer research using the mammary gland of farm animals is concentrating on developing a more efficiently, easy applicable and practically relevant gene transfer procedure.

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SUMMARY

Here, a short overview of the history of gene transfer in farm animals is presented. The general applications of gene transfer technology in animal breeding are summarized. The opportunities of the recombinant protein production in the mammary gland of transgenic farm animals as one of the most successful applications of transgenesis in animal breeding are discussed. Advantages and disadvantages of germline and somatic gene transfer approaches for this purpose are characterized.

BIOTECHNOLÓGIA AZ ÁLLATTENYÉSZTÉSBEN

ZINOVIEVA, NATALIA — BREM, GOTTFRIED

ÖSSZEFOGLALÁS

Az előadás áttekintést ad a gazdasági állatokon végzett génátültetések történetéről, és összegzi a géntranszfer állattenyésztési alkalmazási lehetőségét. Elsősorban a rekombináns fajtákkal előállított nagy biológiai értékű fehérjék tejmirigyben történő szintézisével foglalkozik, ami a transzgenikus szervezeteket, mint bioinkubátorokat használja fel a humán terápiában is alkalmazható fehérjék és nagy hatékonyságú vegyületek bioszintézisére. A tejjel kiváló anyagok előállítása és tisztítása tűnik a biotechnológia egyik legsikeresebb területének. Az őscsírasejt vonalak és a szomatikus génátültetés előnyeit és hátrányait is megvitatja az előadás.

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GENE BANKING IN RARE BREEDS AND SPECIES WHOSE GAMETES ARE DIFFICULT TO CRYOPRESERVE

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INTRODUCTION AND REVIEW OF LITERATURE

Cryopreservation of gametes and embryos is one of the most important technologies supporting advanced animal husbandry, and enabling application of newly developed biotechnological methods. Furthermore, gene banking of rare breeds and/or endangered species is gaining importance as counterbalance to the continuous environmental destruction and subsequent losses of genetic diversity. In several species sperm and embryo cryopreservation is well established. However, in many species gene banking is still very problematic, especially in the area of oocyte and *in vitro* produced or micromanipulated embryo cryopreservation. Novel methods are needed to overcome the limitations, including innovative cryo-procedures and incorporation of new technologies, such as freeze-drying of sperm for room temperature storage, combined with intracytoplasmic sperm injection (ICSI) or nuclear transfer with frozen/thawed somatic cells.

Although intensive research has been conducted on a worldwide scale cryopreservation of oocytes (e.g. female gametes) has not yet achieved sufficient success rates. Oocyte cryopreservation in farm animals, combined with *in*

vitro fertilization and culture would be a valuable tool to maintain genetic diversity; to make a more efficient use of the female genome for breeding purposes, and would provide a suitable recipient cytoplasm source for nuclear transfer experiments. In endangered species, banking of cryopreserved oocytes would facilitate preservation efforts. Cryopreservation of human oocytes, in combination with the currently used human assisted reproductive technologies (ART), would also open new avenues in the treatment of infertility.

The preservation of *in vitro* produced (IVP) or nuclear transfer (NT) embryos is of major significance for the practical application of animal breeding technologies, allowing for long term storage and international commerce of such embryos. Also, gene banking with embryos provides another means to preserve valuable genetics and biodiversity. In human ART methods, besides the well established freezing process for early cleavage-stage embryos, preservation of blastocyst stage embryos is gaining importance.

Since the first successful cryopreservation of mouse oocytes by *Whittingham* (1977), significant increases in efficiency have been reported in many species, mainly due to improved vitrification methods. Since *Lim et al.* (1991) reported that frozen-thawed matured bovine oocytes developed to the blastocyst stage following IVF, several investigators have attempted to cryopreserve bovine oocytes. Oocyte survival, particularly blastocyst development, however, remains low, ranging from 0 to 20% (*Garcia et al.,* 1986; *Fuku et al.,* 1992; *Lim et al.,* 1992; *Otoi et al.,* 1992, 1993, 1995; *Dinnyés et al.,* 1994; *Schellander et al.,* 1994; *Vajta et al.,* 1998). There are only a few studies (*Fuku et al.,* 1992; *Hamano et al.,* 1992; *Otoi et al.,* 1992; *Suzuki et al.,* 1996; *Kubota et al.,* 1998; *Vajta et al.,* 1998) where a small number of pregnancies or births originating from cryopreserved bovine oocytes were reported. According to *Martino et al.* (1996), efforts for the improvement of survival rates have been focused on the cryoprotectants (*Otoi et al.,* 1993; *Dinnyés et al,* 1994) and the freezing (*Lim et al.,* 1991) or vitrification methods used (*Otoi et al.,* 1993; *Vajta et al.,* 1998). Species differences are very important in oocyte cryosurvival. In porcine, oocyte cryopreservation has generally failed to result in progeny despite attempts by several research groups (*Rubinsky et al.,* 1992; *Isachenko et al.,* 1998; *Nagashima et al.,* 1999). Oocyte cryopreservation was successful in several species, including rabbit (*Vincent et al.,* 1989), and human (*Chen,* 1986), however, in non-human primates, including Rhesus monkeys (*Macaca mulatta*) no reports have been published on further development from cryopreserved oocytes. One of the major obstacles in the practical application of oocyte cryopreservation is the potential danger of malformations due to the toxicity of the cryoprotective chemicals and the negative effect of low temperature exposure on different cell components (*Massip et al.,* 1995).

Porcine embryo cryopreservation was considered a very difficult task for several years, until the experiments of *Nagashima et al.* (1995) elegantly proved that removal of the high lipid content, typical for porcine embryos, can drastically improve cryosurvival. Although, micromanipulation methods for removal of lipids were not efficient, nor practical. Recent experiments, however, simplified the procedure by using high-speed centrifugation (*Dobrinsky,* 2002), combined with cytoskeleton modification which subsequently altered membrane flexibility (*Dobrinsky et al.,* 2000). Such improvements in the technology resulted in a

high cryosurvival and several progeny from *in vivo* produced embryos following vitrification.

The effect of cryopreservation is usually evaluated using morphological and *in vitro* developmental characteristics, however, often they don't correlate well with *in vivo* developmental rates. Studies which compare gene activities due to various *in vltro* culture systems, or nuclear transfer methods, have been reported by *Wrenzicky et ai.* (2001) and *Niemann et al.* (2002) for bovine embryos. Similar studies on the gene activation profile of cryopreserved embryos would be beneficial to facilitate the development of methods with a higher potential to result in top quality embryos without relying entirely on pregnancy and birth rates.

Vitrification is a process which prevents ice crystal formation during the cooling of solutions to cryogenic temperatures (*Rall and Fahy*, 1985). Instead of crystal formation, the viscosity of the fluid is increased by many orders of magnitude, giving the fluid the mechanical properties of a solid. The key elements of the method are the high concentration of cryoprotectant and a very rapid cooling and warming rate. The introduction of high concentrations of cryoprotectant into the cell is often damaging. In the past, researchers designing optimal protocols for vitrification of oocytes and embryos, have focused on developing solutions with higher viscosity and cryoprotection by increasing the concentration of the permeating (glycerol, dimethyl-sulfoxide (DMSO), ethylene glycol (EG)) and non-permeating (sugars, Ficoll, proteins, polyvinyl-pirrolidon (PVP)) cryoprotectants, while confronting the problems of higher embryo toxicity (*Vincent et al.*, 1989; *Dinnyés et al.*, 1994).

A novel approach has capitalized on the fact that decreasing the volume of solution reduces the probability of ice crystal formation and promotes successful vitrification (*Vajta et al.,* 1998). A recently developed method (solid surface vitrification, SSV) also applied the strategy of minimizing the volume of the cryopreserved sample by using micro-drops (*Dinnyés et al.,* 2000). This technique vitrifies oocytes in 1–2 μl droplets of vitrification solution by dropping them on a very cold metal surface. The SSV technique has been successfully utilized to vitrify bovine oocytes (*Dinnyés et al.,* 2000) and resulted in higher survival rates than previously reported. In this review recently gathered data on the application of the SSV method in various species and developmental-stages are presented.

In general, cryopreservation of male gametes is more successful than those of females. However, sperm freezing methods still need to be improved to achieve higher survival and fertility rates and to accommodate cryo-sensitivity differences of various species. Furthermore, simplification of the protocols, including those for storage would be highly beneficial. Long-term storage of mammalian sperm cells currently requires temperatures below −110 °C. Consumption of liquid nitrogen or electricity during the storage period makes prolonged storage expensive and vulnerable to low temperature system failures. Alternative storage methods to safely preserve unique genotypes, including transgenic and mutant sperm stocks, would be desirable. Freeze-drying is a method to dehydrate cells so they can be stored at ambient or refrigeration temperatures. Because the process itself does not introduce DNA damage, preserving sperm cells by freeze-drying is feasible and economical. Freeze-drying of mouse spermatozoa combined with ICSI resulted in the birth of live

pups (*Wakayama and Yanagimachi*, 1998). In farm animals, recent experiments using ICSI to inject freeze-dried porcine sperm (*Lee et al.*, 2003) has resulted, for the first time, in high blastocyst development (23/83, 28%), which was not different from that of the controls (21/82, 26%). Transgenic embryos have been produced as well by freeze-dried sperm mediated gene transfer. This has demonstrated the potential application of such approaches in agriculture in the 21st century.

METHODS AND MATERIALS

Details of the SSV method have been described previously (*Dinnyés et al.*, 2000; *Figure 1*). Briefly, oocytes or embryos were washed three times in base medium (species specific) supplemented with 20% serum (from fetal calf or species specific) and then suspended in the SSV equilibration medium (4% EG in base medium) at 34–37 °C for 12–15 min. Oocytes/embryos were rinsed in three small drops (about 25 μL) of SSV vitrification solution (35% EG, 5% PVP, 0.4M trehalose in base medium) on a warming plate at 34–37 °C. The oocytes/embryos were then either processed through the warming solutions (toxicity control) or subjected to the complete vitrification process. In the vitrification process, solution containing 1–15 oocytes or embryos was expelled from the tip of a glass pipette. Then, with a flipping motion the drop was ejected onto the cold surface, which consisted of a metal cube covered with aluminum foil and partially immersed into liquid nitrogen (LN$_2$). Drops varied in size from 1–2 μL.

Fig. 1:. **The solid surface vitrification (SSV) device**
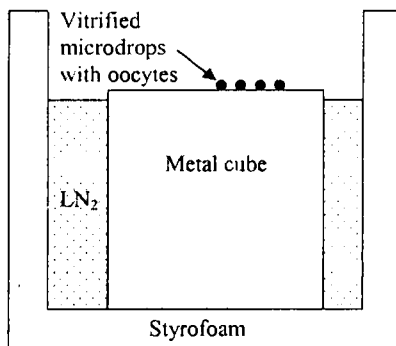
A metal cube covered with aluminum foil is partially submerged into liquid nitrogen. Micro-drops of vitrification solution, containing the oocytes or embryos, are dropped onto the cold upper surface of the metal cube and are instantaneously vitrified

The rinsing and the vitrification were performed in less than 20 sec. The vitrified droplets were moved into cryovials, using nitrogen-cooled forceps, for storage in the gas or liquid phase of a LN$_2$ tank. The SSV droplets were warmed by dropping them, using nitrogen-cooled forceps, into 0.3–0.4 M trehalose in base medium at 37 °C. After 3 min in the solution the oocytes/embryos were washed 2 times in base medium. Finally, oocytes have been used for *in*

vitro fertilization, parthenogenetic activation, or were enucleated and used as recipient cytoplasts for nuclear transfer. Embryos have been further cultured in vitro, or transferred into recipients.

RESULTS AND CONCLUSIONS

Results of experiments with the SSV method are provided below.

Cryopreservation of matured bovine oocytes would aide in gene banking and the improvement of biotechnological methods by providing a continuous source of oocytes for in vitro embryo production or nuclear transfer. The SSV method with vitrified/warmed matured Holstein oocytes resulted in excellent development to the blastocyst-stage after in vitro fertilization, parthenogenetic activation or nuclear transfer (20%, 32% and 27%, respectively; Dinnyés et al., 2000). The same method was applied to Chinese Yellow Cattle oocytes origi- nating from Yunnan prefecture. The Yellow Cattle population of China is declin- ing in numbers, due to recent pressure to use more efficient dairy and beef breeds. Our attempts with the SSV method resulted for the first time in good cryosurvival of these oocytes (80%) and in in vitro blastocyst development from vitrified matured oocytes of this breed (Li et al., 2002). However, the rate of blastocyst development (12%) was far below that achieved with Holstein oo- cytes. Several factors might have contributed to this including the older age of the oocyte donors, and their health and nutritional status. Very likely, the ge- netic origin, especially the Bos indicus background in the Yunnan Yellow Cattle breed has been a significant factor in making cryopreservation of such oocytes more difficult. This example demonstrates that even very advanced vitrification methods need further refinement for applications in gene banking of rare breeds.

Various species might have very different cryobiological characteristics of their gametes and embryos. Finding optimal parameters is a difficult task, and the choice of the cryopreservation method is just one, although very important, element of the process. Goat oocyte and early embryo vitrification by the SSV method have resulted in relatively good survival but no blastocyst development (Begin et al., 2003). Rhesus monkey (Macaca mulatta) oocyte vitrification re- sulted in a low survival rate, yet further cleavage was observed for the first time using the SSV method (Dinnyés, Wei and Ji, unpublished results). In the pig, the survival of in vitro matured oocytes was low (<2%). However, among the surviving oocytes parthenogenetic activation resulted in further development to cleavage stages, including the production of an expanded blastocyst, with a cell number (n=46) not different from that of the controls'. This is the first reported case of achieving such a result in this species (Dinnyés et al., 2001).

In order to find the best combinations of cryoprotectants, technical and bio- logical parameters, mouse is an excellent model species. The most important developmental-stage for practical purposes varies by species. In mouse, pronu- clear-stage has a particular importance, as it is often used for microinjection for transgenic animal production. Our results with the SSV method show that sur- vival of pronuclear-stage embryos was above 90%, and that rates of further development to two-cell stage (80%) and pups born (21%) were high, not differ-

ent from that of the controls (*Bagis et al.,* 2002). Microinjection of vitrified/warmed pronuclear-stage embryos have resulted in birth of healthy transgenic pups.

In vitro production of embryos or their micromanipulation might compromise their viability and cryo-resistance. In cattle blastocyst-stage *in vitro* produced and nuclear transfer embryos have been cryopreserved with great success by the SSV method (*Dinnyés; Kubota and Yang,* unpublished results). In buffalo following nuclear transfer embryos have been successfully vitrified by the SSV method (*Parnpai et al.,* 2001).

Pigs are among the most difficult domestic species for embryo cryopreservation, probably due to the lipid composition of cell membranes. *Table 1* presents data on survival of vitrified *in vitro* produced porcine blastocysts (see details in *Dinnyés et al.,* 2003). While the cryo-survival of *in vivo* porcine blastocysts recently achieved high levels (*Vajta et al.,* 1997; *Dobrinsky et al.,* 2000; *Berthelot et al.,* 2000), very few data are available on the survival of *in vitro* produced pig embryos (*Nagashima et al.,* 2003). The results presented in *Table 1* show that SSV method can be suitable to cryopreserve porcine blastocysts at various developmental-stages, although cryopreservation significantly reduced survival compared to controls (P<0.05). The developmental stage has had no significant (P>0.1) effect on cryosurvival. The main contributing factors for the success might have been the novel vitrification method and the serum-free *in vitro* embryo production system (*Kikuchi et al.,* 2002). Further experiments are needed to increase survival, and to demonstrate the full developmental competence of the vitrified/warmed embryos.

Table 1.

Survival at 24 hr post-warming of vitrified *in vitro* produced porcine blastocysts

Treatment	Expanding blastocysts	Expanded blastocysts
SSV vitrification	21/137 (15%)[a]	24/101 (24%)[a]
Control	17/41 (47%)[b]	22/26 (85%)[b]

Groups with different letters (a, b) within columns differ, P<0.05 (χ^2-test)

The results above from new vitrification methods for the cryopreservation of female gametes and embryos are encouraging. However, further refinements are very much needed to overcome difficulties and achieve practical applications in several species and breeds. Development of the cryo-methods must incorporate molecular biological advances, such as measurements of gene activities, and even genetic modifications of embryos, to improve their cryobiological properties. Introduction of transgenes expressing aquaporins in early embryos (*Edashige et al.,* 2002) is beneficial for better membrane transport of water and cryoprotectants. Complex avenues of gene banking in the 21st century will likely utilize better ovarian tissue preservation; freeze-drying for gamete preservation, together with micromanipulation methods such as ICSI and nuclear transfer with frozen-thawed somatic cells. The low number of oocyte-donor females is one of the bottlenecks of gene banking of rare animals. "Oocyte farming" from ovarian tissues of young animals would capitalize on the great resource of primordial follicles present in the ovary at birth. Even interspecies transfer of ovaries can result in follicle growth and competent oocytes

(*Eppig and Wigglesworth*, 2000). Furthermore, inter-species nuclear transfer already has been successful in gaur (*Bos gaurus*) with bovine (*Bos taurus*) oocytes (*Lanza et al.*, 2000), and in mouflon (*Ovis orientalis musimon*) with sheep (*Ovis aries*) oocytes (*Loi et al.,* 2001). This novel technology might open possibilities to recover genotypes of rare species stored in the form of cryopreserved somatic cells by using recipient cytoplasts from common related species.

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SUMMARY

The review paper describes some new developments in gamete cryopreservation of rare breeds and species in which gametes are difficult to preserve, with special emphasis on vitrification of oocytes and embryos in porcine, rare bovine breeds, goat and rhesus monkey. Furthermore, potential use of somatic cell freezing/nuclear transfer for gene banking and novel methods of preservation of porcine spermatozoa by freeze-drying is discussed. The development of gene banking methods and incorporation of emerging technologies is expected to increase the efficiency of animal husbandry and support wildlife preservation.

RITKA FAJTÁK ÉS NEHEZEN MÉLYHŰTHETŐ GAMÉTÁJÚ FAJOK GÉNBANKI MEGŐRZÉSE

DINNYÉS, ANDRAS — BAGIS, HAYDAR — JI, WEIZHI — KIKUCHI, KAZUHIRO — LEE, JANG-WON — LI, XILONG — NAGAI, TAKASHI — PRESICCE, GIORGIO ANTONIO — SOMFAI, TAMAS — SI, WEI — YANG, XIANGZHONG

ÖSSZEFOGLALÁS

Az utóbbi években az állatok genomjainak a feltérképezése, és a molekuláris genetikai módszerek jelentős mértékben fejlődtek. A humángenetika és a molekuláris biológia területén született új felfedezések jelentős előrelépést tettek lehetővé a gazdasági állatok, így a sertés géntérképezésében. A kutatások legfontosabb területét, a gazdasági szempontból jelentős tulajdonságokat befolyásoló gének és lókuszok (QTL - *quantitativ trait loci*), valamint a kvantitatív tulajdonságok jelzésére alkalmas markergén kutatások adják. A sertés fajban sikerült több értékmérő tulajdonságot befolyásoló, illetve ahhoz kapcsolódó gént felfedezni. Ilyen a növekedéssel, a hátszalonna vastagsággal, a húsminőséggel, a szaporodással és néhány betegség-rezisztenciával összefüggő gén lókusza. Az eddig leírt és feltérképezett gének, többek között a sertés stresszérzékenységét befolyásoló (HAL vagy RIR1), vagy a rendellenes húsminőséget determináló (RN) gének. A szaporasággal (alomnagysággal) összefüggő (ESR, PRLR, RBP4), a növekedéssel és a hátszalonna-vastagsággal kapcsolatos (MCHR), a húsminőséget befolyásoló (PRKAG3) gének, illetve lókuszok, továbbá a betegség rezisztenciáért (FUT1, SLA, NRAMP) felelős gének még azok amelyeket sikerült azonosítani. A különböző tenyésztési programokban ma már sikeresen alkalmazzák az ismert és gazdaságilag fontos értékmérő tulajdonságokat befolyásoló gének vizsgálatát, az ún. markerrel támogatott szelekciós programok (MAS - *marker assisted selection*) keretében. A sertés és egyéb gazdasági állatok géntérképezése, jelentős állami támogatással, intenzíven folyik az USA egyetemein és különböző kutatóhelyein, valamint más országok kutatóintézeteiben. Ezek az új kutatási eredmények növelik a szelekció hatékonyságát, csökkentik a megbetegedések mértékét és fokozzák a sertés általános ellenálló-képességét és ezen keresztül a sertésállomány általános egészségi állapotát is javítják.

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USE OF BIOTECHNOLOGY AND MOLECULAR GENETICS IN SWINE SELECTION PROGRAMS

ROTHSCHILD, MAX F.

INTRODUCTION

Molecular genomic analysis has revolutionized how animal geneticists examine the genetic differences that exist within commercial and exotic pigs. In the past 10 years, efforts have been directed toward the development of genomic maps consisting of anonymous genetic markers and known genes. In addition, comparative genome maps have aided greatly in our search for interesting and potentially useful genes in the pig. The coverage on these genetic maps is now sufficient to allow researchers to search for the causative genes by conducting quantitative trait loci (QTL) linkage analyses. These QTL linkage analyses involve employing a genomic scan where generally F2 or backcross families are used and genotypes are obtained for many (>100) markers evenly spaced across the genome. Several such experiments are underway or recently completed and are beginning to produce interesting and useful results. Candidate gene and comparative mapping approaches have also been successful in identifying major genes affecting several traits. Candidate gene analyses (*Rothschild and Soller,* 1997) are undertaken when a gene is chosen based on the physiology of the trait. This is supplemented by comparative gene analysis that allows researchers to find "positional candidate genes" in the regions associated with possible QTL. The purpose of this paper is to review the recent dis-

coveries of gene mapping and genomics and to forecast future developments
and their applications for genetic improvement in the pig industry.

RESULTS

Status of the genome maps

At present over 4,500 genes and markers have been mapped in pigs
(*Bidanel and Rothschild,* 2002), though not all have been published. Coverage
for the different linkage maps varies, and the average distance between mark-
ers is approximately 3–5 cm. These maps can be combined using markers that
appear in both maps. The physical genetic map in the pig currently consists of
over 800 genes and markers, while the radiation hybrid panel map has over
3,500 markers. Now with the introduction of methods to begin to sequence
genes and the development of ESTs (expressed sequence tags) both the
physical and linkage maps will begin to grow more rapidly and the resulting
comparative map will be enlarged. Despite these concerted efforts, the pig
genome map still pales in comparison to the human and mouse maps.
Complete information on the genetic maps can be seen by going to:
http://www.genome.iastate.edu/pig.

Growth traits

Significant effects of major genes and candidate genes have also been re-
ported (*Bidanel and Rothschild,* 2002). The MC4R (melanocortin 4 receptor)
gene was found to significantly affect growth rate by 7–9% by influencing feed
intake. The MC4R gene maps to chromosome 1 close to a significant QTL.
There is also a significant effect on birth weight of PIT1 (pituitary transcription
factor), which is located on chromosome 13.

The QTL affecting growth traits have been identified on all porcine chro-
mosomes. Genome-wide significant results were obtained for 11 of the 19 por-
cine chromosomes. The most clearly established QTL results were obtained for
chromosomes 1, 4 and 7. The most clearly established QTL logically have the
largest effects on the traits investigated. The QTL located on chromosomes 4
and 7 respectively explained 4–10% and 10–15% of the phenotypic variance of
growth traits. However, significant variation in QTL effects was sometimes ob-
served between studies. This variation may be due to the existence of different
QTL alleles according to the populations studied. Furthermore, QTL effects are
not systematically consistent with breed differences.


Backfat and carcass composition

Back fat thickness QTL (*Fig. 1*) were detected on all porcine chromosomes
except SSC 16 and 17, with genome-wide significant effects on 10 different
chromosomes (*Bidanel and Rothschild,* 2002). Very clear results were also
obtained for the 3 same regions of chromosomes 1, 4 and 7 as for growth traits.

Due to the MC4R effect on feed intake, the candidate gene approach was used and it was shown that variation in this gene is significantly associated with 5-8% differences in back fat and relates to one QTL for back fat thickness on chromosome 1 (*Kim et al.*, 2000). Other regions with back fat QTL include the region carrying the IGF-2 locus at the end of the short arm of chromosome 2 and the central region of chromosome X.

The QTL with significant effects on back fat thickness were also obtained in many other regions (*Malek et al.*, 2000a). Two of them were detected in different regions of chromosome 6.

Fig. 1.: **Candidate genes and quantitative trait loci detected for back fat thickness**
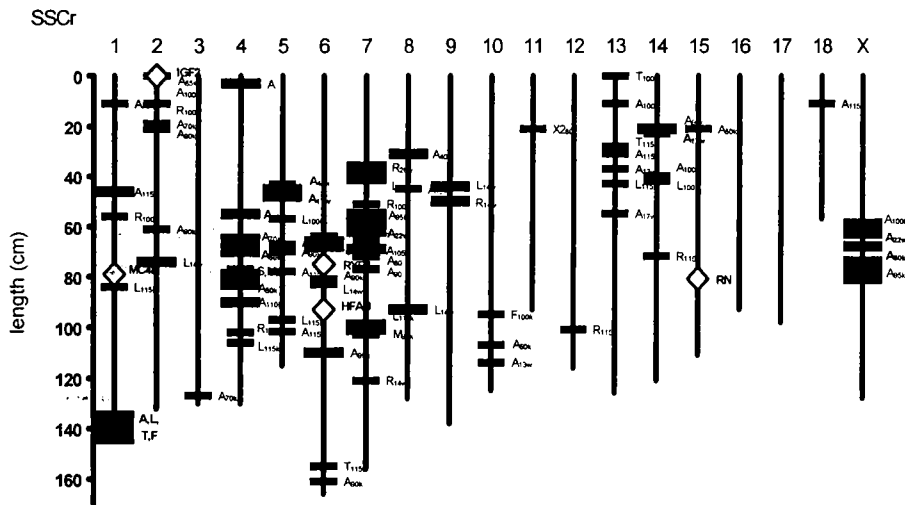
Xy XYz:X=A (average), L (lumbar), R (last rib), T (tenth-rib), S (shoulder), M (mid-back), F (first-rib) back fat -thickness at xx kg (k) or xx weeks (w) of age; Locus names (in bold characters): MC4R=melanocortin-4 receptor locus; IGF2=insulin growth factor 2; RYR1=ryanodine receptor locus; HFAB=heart fatty acid binding protein locus; PIT1=regulatory factor locus; RN="acid meat" locus

Several candidate genes were considered including LEPR, HFABP and MC5R. Similarly, two QTL have been obtained on chromosome 8 and three other significant QTL are localized on chromosomes 5, 9, and 14. One QTL for back fat was also found on chromosome 13, near PIT1.

The chromosome X QTL may have the largest effects measurements based on one experiment, explaining up to 50% of the phenotypic variance of back fat thickness. The QTL located on chromosomes 1, 2, 4 and 7 respectively explain 5–23%, 2–20%, 2–17% and 6–33% of the phenotypic variance of back fat thickness. The other identified QTL explain less than 5% of back fat thickness variability. Of particular importance is whether these results will translate to useful findings in commercial lines. Results in a new experiment by *Evans et al.* (2003) confirmed a number of the fat QTL on chromosomes 1, 3, 4, 6, and 13 in some but not all of the commercial populations.

Meat quality traits

Major genes for meat quality include the HAL or RYR1 gene (*Fujii et al.,* 1991) and RN⁻ (*LeRoy et al.,* 1990; *Milan et al.,* 2000). Chromosomal regions with significant effects on meat quality traits were detected on chromosomes 1, 5, 6, 7, 8, 12, 15, 17 and X (*Bidanel and Rothschild,* 2002). Significant effects on intramuscular fat content or marbling were detected on chromosome 1 (near MC4R), chromosome 6 (near H-FABP), and chromosomes 7 and X. The chromosome 6 and 7 QTL explain 14–18% of the phenotypic variation. Large White or Landrace alleles have unfavourable additive effects as compared to Iberian, Korean native or Meishan alleles. Conversely, Yorkshire alleles have favourable effects as compared to Berkshire alleles for the chromosome 1 QTL, but this QTL only explains 3–4% of the phenotypic variance of intramuscular fat content.

Two QTL located on chromosomes 5 and 15 have significant effects on meat ultimate pH in Berkshire x Yorkshire F2 pigs (*Malek et al.,* 2001b). The QTL located at the end of the long arm of chromosome 5 explains approximately 5% of the phenotypic variance. The same chromosomal region also has suggestive effects on meat colour. The chromosome 15 QTL explains 4–6% of ultimate pH variance and presents favourable, but partly recessive Berkshire alleles. This muscle glycolytic potential QTL is localized in the same region as the RN locus. The RN⁻ mutation (*Milan et al.,* 2000) was not present in the population studied. The observed effect is due to additional mutations inside the RN locus (*Ciobanu et al.,* 2001). Further study has demonstrated that the three mutations when combined into haplotypes produce differences in pH that may be as high as 0.1 pH unit in all breeds except Berkshires in which the differences may exceed 0.2 units. Unlike the RN⁻ mutation, which is essentially only in Hampshires, these three new mutations are in all breeds and this makes them extremely important economically. The QTL for pH at either 45 min or 24 hrs was seen in chromosomes 1, 2, 3, 4, 6, 7, 9, 10 and 13 for the two traits, respectively.

Significant marker-trait associations were detected for meat colour on chromosomes 4, 7, 12, 15 and 17 in several crosses. The chromosome 15 QTL effects are due to the additional mutations found in the *RN* locus (*Ciobanu et al.,* 2001). The chromosome 17 QTL affects both colour score and reflectance measurements. Berkshire alleles are favourable as compared to Large White alleles and explain approximately 4% of the phenotypic variance of both traits. Conversely, no genome-wide significant QTL has so far been detected for water holding capacity, drip or cooking loss. Some suggestive QTL were reported for drip loss on chromosomes 1, 2 and 11 and on chromosomes 4, 6, 14 and 18, for water holding capacity on chromosomes 2 and 13 and for cooking loss on chromosomes 6, 7 and 18 (*Bidanel and Rothschild,* 2002).

A single experiment (*Malek et al.,* 2001b) has carried out a genome scan for meat sensory quality traits, including sensory panel scores. Suggestive QTL were obtained but they correlate well with more objective measures like pH or instron measures of tenderness. In addition, a small but distinct QTL for tenderness was detected in the middle of chromosome 2. Further investigation revealed that Calpastatin (CAST), mapped under the QTL, is a specific inhibitor of calpains, a Ca^{2+}-activated protease family and considered to be the major

cause of initiation of myofibrillar protein degradation. Extensive analysis of the CAST gene revealed several polymorphisms that altered the protein and these had large effect on tenderness (*Ciobanu et al.,* 2002)

Reproduction traits

Given the necessity of larger resource families and the difficulty and time required to obtain information on reproduction traits, it is not surprising that results of QTL scans for these traits are limited (*Bidanel and Rothschild,* 2002). Initial scans have revealed promising results on chromosome 8. Possible QTL for uterine length and ovulation rate were reported, though in different chromosomal positions. A sizable QTL for ovulation rate (+3.07 ova) on chromosome 8 was reported but at a different chromosome 8 location. Later work in the same lab did not confirm this finding. In the French QTL experiment a QTL for increased litter size of one piglet was found in the same location on chromosome 8. The large ovulation rate/litter size QTL on chromosome 8 is of interest as it mapped to the region which is synthetic to the Booroola fecundity gene (a BMP receptor gene) in sheep. Further evidence exists for additional QTL for litter size components on chromosome 8 and other reproductive QTL on chromosomes 4, 6, 7, 13, and 15.

Candidate gene analysis for reproduction has shown considerable merit. Results have clearly demonstrated that the estrogen receptor (*ESR*) is significantly associated with litter size (*Rothschild et al.,* 1996). Estimates of allelic effects vary from 1.15 pig/litter in Meishan synthetics to .42 pigs/litter in Large White lines. These results have not been confirmed by QTL scans using divergent crosses involving Meishan and Large White pigs, perhaps due to small sample sizes or the fact that the ESR gene allele was not segregating in some of the populations involved in the QTL scans. The ESR marker was incorporated successfully into the PIC selection indices for Large White based dam lines, resulting in an increase in the rate of genetic response in its nucleus herds. Furthermore, the increase in average litter size is observed in crossbred products derived from these lines. Other effects have been reported for retinoic acid receptor gamma (RARG), melatonin receptor 1A (MTNRIA), and follicle stimulating hormone beta (FSHB) genes. Iowa State University researchers, working with PIC, demonstrated that the prolactin receptor (PRLR) locus is significantly associated with litter size (*Vincent et al.,* 1998), which was confirmed in two smaller studies. Retinol binding protein 4 (RBP4) was investigated using nearly 2,500 litters and shown to be associated with an increase of about 0.25 pigs per litter (*Rothschild et al.,* 2000). Most candidate gene analyses have involved considerably more sows and litters than the QTL analyses and this might explain the lack of QTL scan confirmation of the regions in which there were candidate gene effects.

Disease resistance and immune response traits

Many disorders (http://www.angis.su.oz.au/Databases/BIRX/omia/) are known to have some genetic influence and the promise of new genomic tools is that the underlying genes might be eventually discovered. To date, QTL scans

for disease resistance or immune response QTL have been limited in pigs. An early exception is work *Andersson* and colleagues (*Edfors-Lilja et al.,* 1998) did to study some immune response parameters. Some immune capacity related QTL have been identified. More recently German scientists have identified regions, in the genome but not genes associated with susceptibility to pseudorabies infection (*Reiner et al.,* 2002).

The existence of a gene responsible for resistance to K88 *E. coli* diarrhea has been known for many years (*Sellwood,* 1979). The gene coding for the K88 *E. coli* receptor in the pig is on chromosome 13 and candidate gene analysis of the region is underway in many labs. Resistance to oedema disease caused by F18 *E. coli* has also been reported and was mapped to chromosome 6 (*Meijerink et al.,* 2000). The work confirmed that a polymorphism in the FUT1 gene is probably the causative mutation for adhesion resistant animals in these breeds. The SLA complex on chromosome 7 has recently been associated with resistance to primary infections with *Trichinella spiralis* but not to resistance to toxoplasmosis. The gene for Natural Resistance Associated Macrophage Protein 1 (NRAMP1), associated with resistance to Salmonella challenge in mice, has been recently mapped to pig chromosome 15 and associations between NRAMP polymorphisms and Salmonellosis in pigs are now being determined (*Bidanel and Rothschild,* 2002). Several other candidate genes are being investigated and many such projects are underway in to discover other genetic abnormalities.

Potential of DNA markers and their use in selection in the livestock industry

Information at the DNA level can help producers, breeders and veterinarians to select for a specific major mutation such as FUT1 resistance or against negative mutations like the negative Halothane allele or RN-allele. The DNA information can also be used to assist in the selection of quantitative traits, called Marker Assisted Selection or MAS (e.g., using ESR B to increase litter size and MC4R to reduce feed intake). Molecular information can increase the accuracy of selection, allow for selection for sex limited traits and allow for selection for traits like meat quality. These approaches have led to a number of genes and markers being used in the swine industry (*Table 1*). More extensive genome scans are underway that will either confirm the regions and lead to the eventual isolation of the gene or genes of interest or will produce conflicting results. Such conflicting results may be the results of haplotype (linked genes) effects, epistasis (interaction) or background genotype effects, or sampling. The size of these experiments will need to be increased or several experimental results will need to be pooled to estimate smaller effects.

Gene expression research

New technical developments continue to provide novel tools that may yield exciting results. In particular, sequencing efforts have now allowed the identification of tens to thousands of individual genes that may be responsible for the traits of interest.

Table 1.

Molecular genetic tests used by the swine industry

Parentage tests	Non exclusive use
HAL	meat quality – non exclusive use
ESR, PRLR, RBP4	litter size – exclusive use (PIC)
KIT	white colour – exclusive use (PIC)
MC1R	red/black colour – exclusive use (PIC)
MC4R	growth and fatness – exclusive use (PIC)
FUT1	oedema *E. coli* F18 – exclusive use (PIC/ITH Switzerland)
RN	meat quality – non exclusive tests (Uppsala, INRA, Kiel)
AFABP, HFABP	intramuscular fat – non exclusive (IPG)
PRKAG3	meat quality – exclusive use (PIC)
CAST	tenderness – exclusive use (PIC)
IGF2	carcass composition – exclusive use (Seghers)
Trade secret tests	several traits – many companies

A list of genetics companies providing routine genotyping in livestock can be seen at:
http://www.genome.iastate.edu/community/genetest.html

These gene projects involve the development of genomic libraries from specialized tissues and then researchers can select expressed sequence tags or ESTs and sequence them. To date several such projects are underway in the pig. Over 100,000 ESTs have been deposited from muscle tissue, reproductive tissue and embryos and from immune response tissue at (http://www.ncbi.nlm.nih.gov/Genbank/GenbankOverview.html) in GenBank or in more specialized databases (http://pigest.genome.iastate.edu/data.html). Many of these ESTs will be mapped so that the comparative map of the pig will advance rapidly. The ESTs can be spotted on microarrays or gene chips so as to study the expression of many genes in parallel. RNA from diseased and healthy animals or other sets of treatments is hybridised to the arrays or chips and expression is compared. The genes that show significant differences between treatments or states are candidate genes that may be important for the trait of interest. A European Community funded project called PathoCHIP (http://www.pathochipproject.com) is such an example.

Future directions

The connection between microarray work and that of QTL has been termed "genetical genomics" and combines arrays, segregation analysis and QTL scan information to identify candidates and crucial steps in biochemical and physiological pathways. Such methods will offer better explanations for understanding genetic control of traits. Development of densely covered SNP maps is likely to follow. Efforts to obtain these SNP maps is progressing and an international effort between Denmark and China to sequence the pig genome is also underway and one is contemplated in the US. These offer great promise for the future of swine improvement.

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SUMMARY

Advances in the fields of molecular genetics and genomics have been considerable over the past several years. Recent discoveries in human genetics and in molecular biology have led to the development of a very useful genetic map of the pig. Several recent quantitative trait loci (QTL) scans and candidate gene analyses have identified important chromosomal regions and individual genes associated with traits of economic interests. In the pig these include QTL for growth and back fat, meat quality traits, reproduction and disease resistance. The causative mutations for porcine stress syndrome (HAL or RYR1) and the Acid meat disorder (RN) for meat quality are now known. Candidate genes for litter size (ESR, PRLR, RBP4), growth and back fat (MC4R), meat quality (PRKAG3), and disease resistance (FUT1, SLA, NRAMP) have been identified. The commercial pig industry is actively using this information and traditional performance information to improve pig production by marker assisted selection (MAS). Research to study the co- expression of thousands of genes is now advancing and methods to combine these approaches to aid in gene discovery are underway. This research will improve pig production, reduce disease and improve the overall healthiness of pigs worldwide.

BIOTECHNOLÓGIAI ÉS MOLEKULÁRIS GENETIKAI MÓDSZEREK ALKALMAZÁSA SERTÉS SZELEKCIÓS PROGRAMOKBAN

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ÖSSZEFOGLALÁS

Az elmúlt néhány évben, a molekuláris genetika és a genomkutatás jelentősen fokozódott. A humán genetika és a molekuláris biológia új felfedezései, a sertések géntérképének a kiteljesedéséhez vezettek. Számos új mennyiségi tulajdonságot meghatározó lókusz vizsgálat (QTL – *quantitative trait loci*) és jelölt gén analízis azonosított jelentős kromoszóma területeket és olyan egyéni géneket, amelyeket sajátos gazdasági érdekekkel társítanak. Sertésekben ezek magukba foglalják a növekedést és hátszalonnát, a húsminőséget, a szaporodást és a betegségekkel szembeni ellenálló-képességet szabályozó mennyiségi, egyéni sajátosságú, lókuszt. A sertés stressz szindróma okozati változásai (HAL vagy RYR1) és a húsminőség savas hús rendellenessége (RN) már ismertek. Az alom méretéért (ESR, PRLR, RBP4), a növekedésért és hátszalonnáért (MC4R), a húsminőségért (PRKAG3), a betegségekkel szembeni ellenállásért (FUT1, SLA, NRAMP) felelős jelölt géneket már azonosították. A kereskedelmi sertésipar aktívan használja ezeket az információkat, de a hagyományos teljesítmény információkat is, hogy *markerekkel támogatott szelekcióval* (MAS – *marker assisted selection*) fejlessze az ágazat hatékonyságát. A kutatás több ezernyi gén együttműködésének a tanulmányozásával jól halad, és a különböző megközelítések kombinálása a gének felfedezésének segítségére is folyamatban van. Ez a kutatás segíteni fogja a sertéstenyésztést, csökkenti a betegségeket, és javítani fogja a sertések általános egészségét az egész világon.

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Faber, David C. was born on a grain and livestock farm in north central Illinois (USA) and received the BS and DVM from the University of Illinois. He joined a large food animal practice in northwest Iowa, began doing embryo transfer work and founded Trans Ova Genetics in 1980. Dr. Faber serves as the president of Trans Ova Genetics, one of the leading bovine embryo transfer companies in the U.S. In addition to embryo collection, cryopreservation, and transfer, Trans Ova Genetics offers services in in vitro embryo production, ultrasonography, oocyte retrieval and micromanipulation, and the production of transgenic farm animals capable of expressing pharmaceutical proteins in their milk. He is a member of the Society for Theriogenology, International Embryo Transfer Association, American Association of Bovine Practitioners, and the Academy of Veterinary Consultants.

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APPLICATIONS OF BIOTECHNOLOGY IN CATTLE

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History of reproductive biotechnology

The improvement of cattle genetics through reproductive technology has been very significant in the last two decades. The commercialization of artificial insemination (AI) was extensive around the world after researchers perfected the methods to cryopreserve semen. In this manner, semen could be stored and delivered to any piace that needed it (*Polge et al., 1949; Smith and Polge, 1950; Nagase et al., 1964*). After that, the AI global market focused on developing elite sires, selected through progeny tests, with traits that satisfied the needs of beef and dairy producers. During this time, AI helped to improve the management of the herds by synchronizing the calving season in agreement with the necessities of each establishment. This was achieved with the implementation of estrus, follicular dynamics and ovulation synchronization programs through hormonal ovarian function control. The female contribution to genetic progress was achieved with the embryo transfer technique for non-surgical embryo collection (*Elsden et al., 1976*) and the *in vitro* maturation (*Leibfried and First,* 1979), fertilization and culture of bovine oocytes (*Brackett et al., 1982;*

Brackett, 1983; *Eppig and Schroeder,* 1986; *Kane,* 1987; *Leibfried-Rutledge et al.,* 1987; *Leibfried-Rutledge et al.,* 1989). In the same manner as frozen semen, embryo freezing allowed for the global commercialisation of animals with high genetic qualities (*Wilmut and Rowson,* 1973; *Mazur,* 1980; *Lehn-Jensen,* 1984; *Leibo,* 1984; *Leibo,* 1988; *Massip et al.,* 1987; *Niemann,* 1991; *Rall,* 1992; *Voelkel and Hu,* 1992). With the ability to produce *in vitro* embryos and to achieve pregnancies (*Lu et al.,* 1987; *Xu et al.,* 1987; *Goto et al.,* 1988; *Fukuda et al.,* 1990), genetic manipulation of embryos became feasible. The first calves produced by nuclear transfer (NT, or cloning) were illustrated by publication in 1987 (*Prather et al.,* 1987). After a decade of work, the first cloned sheep by NT of adult somatic cells was achieved (*Wilmut et al.,* 1997). At present, several commercial companies have begun to offer NT, but this technology still has low efficiency (*Westhusin et al.,* 2001; *Renard et al.,* 2002). The primary purpose of NT is to produce transgenic animals and to produce genetic copies of high value animals. The first experiences were carried out with sperm-mediated gene transfer and pronuclear microinjection. At present, many laboratories are carrying out studies using other techniques, like viral-mediated, retrovirus-mediated and sperm-mediated gene transfers (*Wall,* 2002). The commercial application of transgenic livestock production will first be in the biopharmaceutical and biomedical areas. Future applications include the development of disease resistant animals or animals with particular qualities (i.e. transgenic sires that produce females or males only, "sex bulls"). Commercial applications will also include improving meat and milk quality, which will increase the efficiency of the livestock industry.

Commercial IVF

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 (*Callensen et al.,* 1987). 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 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 centres 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 (*Kruip et al.*, 1994; *Looney et al.,* 1994; *Hasler et al.,* 1995; *Galli and Lazzari,* 1996; *Schmidt et al.,* 1996; *Bols,* 1997; *Guyader Joly et al.,* 1997; *Larsson,* 1998; *Bousquet et al.,* 1999; *Eikelmann et al.,* 2000; *Guyader Joly et al.,* 2000; *Perez et al.,* 2000; *Blondin et al.,* 2002; *Ferré et al.,* 2002; *Reis et al.,* 2002). Overall results with problems cows are presented in *Table 1.*

Table 1.

Overall OPU-IVF results with problems 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)

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 (*Holm et al.,* 1999) 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.

Table 2.

Summary OPU-IVF results with problems 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]

Value 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. Value with different superscripts in the same column differ (P<0.05)

Table 4.

Breed performance in OPU-IVF problem cows

Breeds	No. donors	OPU sessions	Oocytes	Oocytes/ session	Embryos/ session	Embryos (%)	Pregnancy rates, %
British	170	1872	12945	6.9	1.10	2085 (16.1)	924 (44.3)
European	192	2097	15185	7.2	1.30	2767 (18.2)	1365 (49.3)
Indian	94	828	11536	13.9	1.40	1156 (10.0)	658 (57.0)
Asian	18	51	372	7.3	0.86	44 (11.8)	27 (61.4)
XX	6	114	617	5.4	1.40	155 (25.1)	78 (50.3)
American	7	28	191	6.8	1.30	36 (18.8)	10 (27.8)
Dairy	237	1616	9583	5.9	0.87	1405 (14.7)	618 (44.0)

All these embryos were transferred fresh due to the poor results obtained with frozen *in vitro* embryos. This higher sensibility (*Leibo and Loskutoff,* 1993; *Pollard and Leibo,* 1993; *Palasz and Mapletoft,* 1996; *Massip,* 2001;) would be due to the culture conditions or fertilization protocol and would produce modifications in the *in vitro* embryo (*Hyttel et al.,* 1989; *Fukui et al.,* 1991; *Suzuki et al.,* 1991; *Shamsuddin et al.,* 1992; *Van Soom and de Kruif,* 1992; *Greve et al.,* 1993; *Pinyopummintr and Bavister,* 1994; *Poilard and Leibo,* 1994; *Trounson et al.,* 1994; *Carolan et al.,* 1995; *Massip et al.,* 1995; *Wright and Ellington,* 1995; *Holm and Callensen,* 1998; *Lonergan et al.,* 2001ab; *Rizos et al.,* 2002).

There were statistical differences in percentages of embryo development and pregnancy rates between treatments. The FSH application increased the oocytes number, transferable embryos per OPU session and oocytes quality. Recent evidence shows the necessity to correct the superstimulatories treatment scheme, which include the control of follicular development and complete developmental competence oocytes (*Blondin et al.,* 2002).

Many factors influence the efficiency of IVF technology, but the main ones 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 the milk market 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 (*Hohenboken,* 1999). Several methods have been used to reach this objective which is presented in *Table 5.*

Table 5.

Different methods of sexing

Sexing	Method	References
Semen	DNA content	*Morrell,* 1991; *Blecher et al.,* 1999; *Hendriksen,* 1999; *Johnson and Welch,* 1999; *Seidel and Johnson* 1999; *Munster et al.,* 1999
Embryo	Biopsy and PCR, fluorescence in situ hybridization	*Bredbacka et al.,* 1995; *Seidel,* 1999; *Lonergan et al.,* 2001
Fetus	Ultrasonography at 60–90 days of gestation	*Curran and Ginther,* 1991

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) or 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 (*Seidel,* 1999).

At present in the United States, there exists one commercial company that offers sexed semen (XY, Inc., Fort Collins, Colorado) and 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 (*Johnson and Welch,* 1999). The 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 (1–1.5x10^6 sperm) and high dose (3x10^6 sperms) units of frozen sexed semen deposited in the uterine body (*Seidel and Johnson,* 1999). Similar results were obtained by Goyaike Company with the same sexed semen technology in Ar-

gentina (Brogliatti et al., 2002). In IVF, it is feasible to reach 18–26% of embryo development with frozen sexed semen (Lu et al., 1999; Lu et al., 2001).

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 edge of commercial application. To date, our attempts to use separated sperm populations with Artificial Insemination coupled with superovulation and in vivo embryo transfer, have produced very low fertilization rates. Superstimulated beef and dairy donors may fail to transport sperm efficiently to the site of fertilization in the oviduct (Seidel and Johnson, 1999).

The commercial application of separated semen coupled with IVF appears to provide the most logical and first commercial application for separated semen. The inherent high cost of separated sperm fits well into commercial IVF schemes. The potential to separate frozen-thawed sperm would provide an advantage to IVF production of embryos when compared to standard in vivo collection and transfer of embryos.

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 6.

Somatic cell cloning

Cloning in cattle: The most acclaimed example of animal cloning is, of course, the report by Wilmut et al. (1997) the first to demonstrate that cloning of adult mammals was possible.

Table 6.

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

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

Value with different superscripts in the same row differ (P<0.05)

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 (Campbell et al., 1996; Stice et al., 1998; Baguisi et al., 1999; Wells et al., 1999). Currently, most cloning efforts are focused on the production of transgenic animals by utilizing genotypes that are defined by a particular genetic modification (Wall, 1996; Murray, 1999; Turner, 1999; Brink et al., 2000). However, there is also considerable demand for cloning animals that

have high genetic or resale value. Included in these groups are bulls with high semen sales potential and cows with significant embryo revenue production. In addition, show cattle may also be selected for cloning (*Stice et al.,* 1998).

There are more laboratory groups working on cattle cloning than in all other species combined. Success in cloning cattle is in part due to the application of assisted reproductive techniques in cattle which have been employed commercially for several years. Successful and repeatable procedures for *in vitro* oocyte maturation, *in vitro* fertilization and *in vitro* embryo culture are well established in cattle. Each represents a key step in the cloning process. In addition, there is potential to access large number of oocytes from abattoirs at a relatively low cost.

The efficiency of cloning cattle by nuclear transplantation is extremely variable (*Wells et al.,* 1999). 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.

To date, approximately 3643 cloned embryos have been transferred into 1898 recipients. An average of 1.9 embryos per recipient were transferred. Recipient pregnancy rates (as determined via ultrasonography at 40 days) have ranged from 0–86%. Calving rates have ranged from 0–32%. Calf survival has ranged from 0–100%.

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. Certain cell lines, manipulations, and treatments have had extremely high mortality. The leading causes of mortality include respiratory distress, birth defects, non-viable calves, and enteritis (Clostridium sp).

Commercial application of cloning in cattle is dependent on three factors.
— Economics,
— Societal values,
— Animal welfare,
— Environmental concerns,
— Consumer acceptance,
— Regulatory agencies,
— Animal health,
— Food safety.

1) Economics

Certainly the primary driver in all assisted reproductive technology is 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 viability are the major hurdles to widespread application of the technology (*Behboodi et al.,* 1995; *Wilson et al.,* 1995; *Garry et al.,* 1996).

2) Societal values and regulatory impact on commercialisation

Commercial utilization and the gap in scientific knowledge of their risks pose extensive concerns. The trigger for regulatory consideration has traditionally been at the point of transfer of a technology to commercial application. Commercial companies have moved more quickly to implementation than regulators expected.

The crisis caused by BSE and foot and mouth disease has accelerated public interest in food safety, and led to a distrust of regulatory agencies. The birth of Dolly has tended to polarize public opinion on the application of biotechnology in agriculture. Historically, most technology introductions have been met with some scepticism. 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 piacing 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 (*Evans,* 1999).

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.

Bio medical applications

A revolution in the treatment of disease is taking shape due to new therapies based on human recombinant proteins. The ever-growing demand for such pharmaceutical proteins is an important driving force for the development of safe and large-scale production platforms. Since the efficacy of a human protein is generally dependent on both its amino acid composition as well as various

post-translational modifications, many recombinant human proteins can only be obtained from mammalian cells. Mammalian cell culture systems are often used for expression. This approach is generally known for limited production capacity and high costs. In contrast, the production of (human) recombinant proteins in milk or blood of transgenic farm animals, particularly cattle, presents a safe alternative without the constraint of limited protein output (*Wright et al.,* 1991; *Ziomek,* 1998).

Unlike rodents and smaller dairy producing mammals, transgenic cattle are highly efficient "factories" capable of producing large amounts of valuable protein in their blood or milk. In addition, transgenic cattle are bom and raised in a controlled environment where every variable is accounted for, thereby significantly decreasing the risk of disease transmission. Using multiple "firewalls" and strict quality control systems, can ensure delivery of a stable intermediate form of bio-protein.

In our laboratory, early attempts at the production of transgenic bovines involved microinjection of a DNA construct (which coded for the desired protein and expression site), directly into one-two cells bovine embryos. This was extremely inefficient with less than 1 % of the resulting offspring being fully transgenic. Typically, transgenesis rates have been reported from 0–10% for cattle, with rates usually being <5% (*Hammer et al.,* 1985, *Hyttinen et al.,* 1994).

The birth of Dolly provided for a new approach in the production of transgenic bovines.

Manipulation of donor cells *in vitro*, followed by somatic cell cloning has led to a high percentage of transgenic offspring.

Strategies for founder production usually involve the production of 3–4 founder lines from which eventual production animals can be selected. Copy number and positional effects have produced variability in the expression of the desired protein. Today, candidate cell lines are further screened to look at copy number prior to use as donor cells in cloning. Ideally, candidate cell lines could be screened for expression and quality of the desired protein.

The production of bio proteins from the milk or blood of transgenic animals requires an orchestrated application of several core competencies.

— Bio Protein core competencies,
— Quality assurance, quality control, and regulatory,
— Molecular biology,
— Cell biology,
— Cloning,
— Embryo transfer,
— Bio Protein cattle management,
— Clarification, purification,
— Down stream processing,
— Clinical trial collection,
— Herd scale up,
— Management of Bio Protein farms,
— Marketing of the resulting Bio Protein.

At present, no single company possesses all of these capabilities and intellectual property. Pharmaceutical companies are interested in Bio Proteins in a stable form. Due to this perceived demand, we have attempted to develop nec-

essary core competencies and relationships to be the "bridge" between technology and pharmaceutical starting material.

While considerable time has been spent discussing embryo technologies such as nuclear transfer, this would be incomplete without some general discussion about regulatory considerations when producing bio proteins from cattle. The one overlapping core competency necessary for commercialisation is regulatory knowledge and experience.

This discipline requires the implementation of standard operating procedures and documentation. The guiding adage is "if you did not document it, you did not do it". This represents a crucial paradigm shift from agriculture and research.

Animals used as bioreactors have significant advantages in economical production of protein. However the disadvantages include the potential for prion, viral and bacterial pathogens. In addition, feedstuffs allow for the introduction of undesirable chemicals such as herbicides and pesticides in the source material.

Documentation of animal origin, animal health, feedstuffs, and management practices are critical to any commercialisation strategy. Successful implementation of these procedures, coupled with management of the master seed bank will contribute to the production of a safe, efficacious, and consistent product.

Commercialisation 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 comes from the application of combinations of these technologies.

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SUMMARY

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.

Commercialisation 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 fibre.

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 commercialisation will benefit all of the above stakeholders, and provide a safe efficient supply of food and pharmaceuticals.

A BIOTECHNOLÓGIA ALKALMAZÁSA
A SZARVASMARHA-TENYÉSZTÉSBEN

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ÖSSZEFOGLALÁS

Az állat-biotechnológia gyorsan fejlődő tudományos és információ technikai tudományágak egyre növekvő gyűjteményét jelenti. A szarvasmarhafélék sok lehetőséget adnak ezen tudományágak hasznosítására.

Manapság a szarvasmarha biotechnológia az üzleti alapokra helyezése, két módon történik. Az egyik mód, az állatok orvosi célokra való felhasználását jelenti. Nagyon kevés vállalat van az összes lényeges szakértelemnek és szellemi tudásának a birtokában ahhoz, hogy áthidalják a laboratórium és végtermék közti szakadékot. A másik mód az állatok tenyésztése, élelmiszertermelés céljából.

A mesterséges termékenyítés, az embrió átültetés, az in vitro megtermékenyítés, a klónozás, a génátültetés és a genomkutatás mind része annak az eszközkészletnek, amelyet jelenleg és a jövőben alkalmazni fognak. Ezek önmagukban is nagyon hatásosak eszközök, amelyek képesek a termékenységet jelentősen javítani. Ezen technológiák kombinálása információs rendszerekkel és adatelemzéssel egybekötve, még jelentősebb változásokat fognak előidézni a következő évtizedekben.

Az állat biotechnológia bármely ágának kereskedelmi felhasználása, a szabályozás gondos áttekintését és a társadalmi érdekek betartását követeli. A biotechnológia ipari infrastruktúrájának gondos kialakítása is fontos. A növény biotechnológiában tevékenykedő kollégáink segítettek rávilágítani néhány csapdára, amit a módszerek rejtenek magukban.

Összegezve, ma jelentős ismereteink vannak, amelyek gazdag lehetőségekkel ruházzák fel a társadalom tagjait, a kereskedelmi társaságokat és a szarvasmarha tenyésztőket. A biotechnológia sikeres üzleti alapokra helyezése segíteni fogja a felsorolt érdekelteket a magas színvonalú termelésben, és az élelmiszerek valamint a gyógyszerek biztonságos és hatékony kínálatát fogja megteremteni.

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IN VITRO EMBRYO PRODUCTION IN PIGS

KIKUCHI, KAZUHIRO

INTRODUCTION

An *in vitro* embryo production (IVP) system includes *in vitro* maturation (IVM) and *in vitro* fertilization (IVF) of oocytes, and also *in vitro* culture (IVC) of the fertilized oocytes or zygotes. The establishment of a porcine IVP system enables us to generate viable embryos as well as *in vivo* derived embryos with less cost and in less time, and will contribute to research in reproductive physiology, agriculture, and biotechnology, including cloning and transgenesis in pigs. The *in vitro* developmental competence or viability of porcine IVM/IVF oocytes to the blastocyst stage was first confirmed and reported by *Matiolli et al.* (1989). Further, piglets have been born from IVM/IVF embryos after IVC to the two- to 4-cell stage (*Yoshida et al.,* 1993). Since then, some laboratories have succeeded in producing piglets from cleaved embryos at the two- to 4-cell stage after IVM/IVF and IVC for 24 to 36 h (*Funahashi et al.,* 1996,1997), and from 8-cell to morula-stage embryos after IVM/IVF and IVC for 96 h (*Day et al.,* 1998). However, it was only quite recently that viable piglets were generated after transfer of IVP embryos at blastocyst stage (*Marchal et al.,* 2001; *Kikuchi et al.,* 2002a). Here, we discuss the developmental ability of porcine embryos produced by this improved IVP system and the utilization of this technique in terms of biotechnology.

Current status of the IVM/IVF system in pigs

IVM systems for porcine follicular oocytes have been improved in recent years, emphasizing the importance of the redox state and glutathione content in relation to cysteine in the maturation medium (reviewed by *Nagai,* 2001) and

the synchronization of early meiosis in oocytes (*Funahashi et al.,* 1997). Oxygen tension during IVM is also an important factor in cytoplasmic maturity for *in vitro* development to blastocysts (*Kikuchi et al.,* 2002a). When cumulus-oocyte complexes were matured *in vitro* under 5% O_2 or 20% O_2, fertilized *in vitro* under 5% O_2, and subsequently cultured under 5% O_2, there were no significant differences in blastocyst formation rates on Day 6 between the 5% O_2 and 20% O_2 conditions (19.9% and 14.0%, respectively). However, the quality of the blastocysts, as evaluated by total cell number, was better after IVM under 5% O_2 than under 20% O_2 (mean cell number 43.5 and 37.8, respectively).

Improvements in many aspects of IVF procedures have been made over many years, but polyspermic fertilization during IVM has remained an unsolved problem in porcine IVP (reviewed by *Nagai,* 1996). Some of the polyspermic fertilized oocytes can develop to the blastocyst stage (*Han et al.,* 1999), so careful attention must be paid to the relationship between polyspermy and embryonic development.

Improvement of IVC of porcine IVM/IVF oocytes

The developmental competence of IVP embryos before the blastocyst stage (two-cell to morula stage) to the viable piglet has already been confirmed by embryo transfer experiments, but successful piglet production after blastocyst transfer was not reported until 2001. Thus although IVP blastocyst production itself was first successful in 1989, viable blastocyst production was not established until more than 10 years later. In addition to the improvement of IVM/IVF procedures, improvement of the IVC procedure seems to be the more important factor needed to obtain viable blastocysts. North Carolina State University (NCSU) solutions containing taurine and hypotaurine (NCSU-23) or sorbitol (NUCU-37) were originally established for culture of porcine *in vivo* derived embryos (*Petters and Wells,* 1993). These innovations were introduced to the IVC of porcine IVM/IVF oocytes but resulted in the problem of low embryo quality. For example, the total cell numbers of the blastocysts were reported to range from 30 (*Abeydeera and Day,* 1997) to 37 (*Wang et al.,* 1997), although an advanced IVC system had been expected. To examine the developmental competence of IVM/IVF oocytes, we conducted the following two experiments (*Kikuchi et al.,* 1999; *Kashiwazaki et al.,* 2001).

Experiment 1: We examined the effect of the duration of IVC on ability to develop to the fetal stage or to term. Porcine cumulus-oocyte complexes were matured *in vitro* under 5% O_2, fertilized *in vitro* and subsequently cultured under 5% O_2 for 0, 24, or 48 h in NCSU-37 solution. The embryos were then surgically transferred to the oviducts of recipients in which estrus had been synchronized with eCG and hCG. On the 29th day post-IVF, the uteri of some of the recipients were surgically examined for pregnancy, then the pregnant females were hysterectomised in order to examine the number and weight of the fetuses. Rates of development to fetuses of IVM/IVF oocytes cultured for 24 and 48 h were significantly lower (1.7% and 2.0%, respectively) than those of IVM/IVF oocytes that did not receive IVC (6.7%). However, the weights of fetuses (1.0 to 1.2 g) did not differ among the experimental groups. The remaining recipients were examined for pregnancy with an ultrasonic pregnancy detector and the

pregnant females were allowed to go to term. Some recipients that had received embryos cultured for 0 or 24 h farrowed healthy piglets, but no farrow was obtained from embryos cultured for 48 h before the transfer. The results indicate that the viability of porcine IVP embryos is decreased by IVC after IVF.

Experiment 2: We examined the development of IVM/IVF oocytes to the blastocyst stage under *in vivo* or *in vitro* conditions. IVM/IVF oocytes were generated by the same procedure as that used in Experiment 1 and transferred to the oviducts of synchronized recipients just after IVF. More than 70% of the blastocysts recovered 6 days after transfer had developed to the hatched blastocyst stage, with a mean cell number of 181.5 per blastocyst — approximately the same number found in *in vivo* blastocysts before hatching (*Davis*, 1985). In contrast, when IVM/IVF oocytes were cultured *in vitro* for 2 days and transferred to the oviducts of recipients, or cultured *in vitro* for 6 days without transfer to oviducts, most of the blastocysts recovered were still enclosed by *zona pellucidae*. The mean cell numbers of blastocysts obtained from IVM/IVF oocytes cultured for 2 days *in vitro* plus 4 days in the oviducts or for 6 days *in vitro* decreased significantly to 58.2 and 38.4, respectively, compared with that of blastocysts obtained from IVM/IVF oocytes cultured in the oviducts for 6 days. These results suggest that porcine IVM/IVF oocytes have high potential for developing to the blastocyst stage — equal to that of *in vivo* matured oocytes — and that the IVC conditions (NCSU-37) used for IVM/IVF oocytes in our present study were suboptimal. We conclude that an improved IVC system for IVM/IVF oocytes is needed.

Next, we modified the IVC procedure to establish reliable IVP procedures for porcine blastocysts and to examine the ability of the blastocysts to develop to term after transfer to recipients (*Kikuchi et al.,* 2002a). Porcine cumulus-oocyte complexes were matured *in vitro* under 5% O_2, fertilized *in vitro*, and subsequently cultured under 5% O_2 in 1) NCSU-37 solution supplemented with glucose (IVC-Glu; original NCSU-37) from Day 0 (the day of IVF) to Day 6; 2) IVC-Glu from Days 0 to 2, then NCSU-37 solution without glucose but supplemented with pyruvate and lactate (IVC-PyrLac) from Days 2 to 6; 3) IVC-PyrLac from Days 0 to 2, then IVC-Glu from Days 2 to 6; or 4) IVC-PyrLac from Days 0 to 6. When IVM/IVF oocytes were cultured in IVC-PyrLac from Days 0 to 2 and subsequently in IVC-Glu from Days 2 to 6 (Regimen 3), the rate of blastocyst formation (25.3%) and the number of cells (48.7) were higher than the other rates (5.8% to 18.1%) and numbers (35.4 to 37.1) with the other supplement regimens (Regimens 1, 2, and 4). We then prepared conditioned medium (CM) from the culture of porcine oviductal epithelial cells for 2 days in IVC-PyrLac and evaluated its effect on development to the blastocyst stage. Cultivation in CM for the first two days, followed by IVC-Glu for a further 4 days, had a significantly greater effect in increasing the number of cells in the blastocyst (58.3) than did in the unconditioned control medium (IVC-PyrLac) for the first 2 days followed by IVC-Glu for 4 days (48.4). Finally, we evaluated the ability of blastocysts generated after IVC in CM to develop to term. When Day 5 expanding blastocysts (mean cell number 49.7) were transferred to an estrus-synchronized recipient (50 blastocysts per recipient), the recipient remained pregnant and farrowed 8 normal piglets. Furthermore, when Day 6 expanded blastocysts (mean cell number 80.2) were transferred to two estrus-synchronized recipients,

both gilts remained pregnant and farrowed a total of 11 piglets. These results suggest that an excellent piglet production system can be established by using this modified IVP system, which produces high-quality porcine blastocysts.

Application of porcine IVP system for new technologies

We expect that this modified IVP system can be utilized not only in the field but also in some of the newly developing biotechnologies.

Important technologies: Embryo cryopreservation and non-surgical embryo transfer are technologies that are still undeveloped in the field of pig reproduction. The arrival of these technologies has been anticipated for many years, especially in the breeding area, but they are still incomplete. One of the main reasons is that not enough *in vivo* derived embryos can be produced economically for the experiments. An average of 27.2 embryos can be obtained from a donor prepubertal gilt superovulated by hormonal administration (*Brüssow et al.,* 2000). In addition, reactivity to the hormones differs among donor animals. The use of *in vivo* materials is thus not efficient enough to complete these experiments. In contrast, several hundreds of embryos can be obtained at a time by IVP, and we expect that these embryos will be used to compete and establish these new technologies.

Cryopreservation of porcine embryos in liquid nitrogen by slow freezing has succeeded, and piglets have been obtained after transfer to recipients (*Kashiwazaki et al.,* 1991). Vitrification of *in vivo* derived blastocytes has also succeeded in pigs (*Kobayashi et al.,* 1998). Vitrification of IVP blastocysts has been conducted in many laboratories, but because of the low quality of IVP embryos no one has yet succeeded in producing piglets after the transfer of vitrified embryos. The blastocysts produced in our laboratory can be vitrified, and some of the warmed ones have survived *(Dinnyes et al.,* 2003): according to the report, the survival rate at 24 h post-warming of vitrified Day 5 expanding blastocysts was 15%, and of Day 6 blastocysts, 23% to 24%. However, these vitrified blastocysts have not yet been carried to term after transfer to recipients. The reason for this is probably the low tolerance of porcine IVP embryos to cryopreservation and their high content of cytoplasmic lipid droplets. Porcine oocytes and embryos contain more lipids than those of other mammals, and it is known that the removal of lipid from the embryos increases their tolerance to cryopreservation (*Nagashima et al.,* 1995). Because the lipid droplet distribution differs between IVP and *in vivo* derived embryos (Kikuchi et al., 2002b), differences in tolerance to cryopreservation should also be examined. In addition, membrane stability or microfilament alterations (*Dobrinsky et al.,* 2000) may also be important factors in successful cryopreservation.

Surgical embryo transfer is an established procedure. In contrast, non-surgical transfer is only just now beginning to be used, despite the fact that the first successful pregnancy by this technique was reported more than 30 years ago (*Polge and Day,* 1968). *Reichenbach et al.* (1993) showed that 25 to 40 *in vivo* derived embryos at the 8-cell to hatched blastocyst stage were required per recipient for the successful production by non-surgical transfer, where the mean number of piglets was 5. This inefficacy seemed to be related to the anatomical complexity of the uterus and cervix in pigs. To improve these rates, special

flexible instruments may be required for transfer. For this purpose, IVP embryos as well as *in vivo* derived embryos should be used as materials.

New biotechnology: Our advanced porcine IVP system is expected to be utilized in cloning and intracytoplasmic sperm injection (ICSI) for reproduction and gene modification.

Successful pig cloning using *in vivo* matured (*Onishi et al.,* 2000; *Polejaeva et al.,* 2000; *Bondioli et al.,* 2001; *DeSousa et al.,* 2002; *Boquest et al.,* 2002) and IVM oocytes (*Betthauser et al.,* 2000; *Lai et al.,* 2002; *Yin et al.,* 2002) as recipient oocytes has been reported. At our institute, after enucleating of a donor nucleus, injection of the somatic nucleus, and cultivation to the two- to 4-cell stage, embryos were encapsulated within sodium alginate to avoid loss in the recipient oviducts. From the transfer of a total of 1344 embryos, 174 embryos at the morula or blastocyst stage were collected. They were re-transferred to 4 estrus-synchronized recipients: one of them became pregnant and farrowed one cloned piglet (*Iwamoto et al.,* 2003). These reports confirm that, in pigs, IVM oocytes as recipient oocytes have the ability to develop to term.

Successful piglet production after ICSI has been reported after the injection of *in vivo* matured oocytes with boar spermatozoa (*Koibe and Holtz,* 2000; *Martin,* 2000). Recently, our lab has succeeded in producing piglets after the transfer of IVM oocytes, each injected with a boar sperm head (*Nakai et al.,* 2003). The use of this technique seems to be applicable not only to reproduction using boar sperm — which show poor motility after thawing — cryopreserved as genetic resources, but also in biotechnologies such as sperm-mediated gene transfer. The efficacy of sperm-mediated gene transfer has already been confirmed in pigs (*Lavitrano et al.,* 1997). In mice, the combination of ICSI and sperm-mediated gene transfer has already been reported (*Perry et al.,* 1999).

Cloning and ICSI technologies using IVM oocytes are now expected to be used to produce transgenic and knockout pigs because they are less costly and time-consuming than use of *in vivo* derived oocytes. However, their efficacy in the development to blastocyst stage seems to be low. Although the reasons for the low developmental competence of cloned and ICSI embryos are unclear at present, mechanical damage caused during micro-manipulation is the most probable cause. Further, unphysiological oocyte activation processes (for example, by electric pulses just after the micro-manipulation) may affect developmental ability and should also be considered. Further research to maintain developmental ability after the manipulation of IVM oocytes is needed.

CONCLUSION

The established porcine IVP system now includes new technologies for reproduction and gene manipulation and is ready for practical application, although further improvements are still required.

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SUMMARY

The establishment of *in vitro* embryo production (IVP) system in pigs enables us to generate viable embryos with a quality equal to that of *in vivo* derived embryos. This technology contributes not only to developments in reproductive physiology and agriculture but also to biotechnologies for producing cloned or genetically modified pigs. The birth of piglets from *in vitro* matured and fertilized embryos at the two- to 4-cell stage was first achieved about 10 years ago, but it was only quite recently that piglets were produced after the transfer of IVP blastocysts. This improvement to the blastocyst stage of the *in vitro* culture system after *in vitro* maturation and fertilization can be expected to play a part in the development of an advanced IVP system. Here, we discuss the developmental ability of porcine embryos produced by our improved IVP system and the utilization of this technique in the new biotechnology.

IN VITRO SERTÉSEMBRIÓ-ELŐÁLLÍTÁS

KIKUCHI, KAZUHIRO

ÖSSZEFOGLALÁS

A laboratóriumi viszonyok között előállított embrió (*in vitro embryo production* — IVP) rendszer létrehozása sertések esetében lehetővé teszi, hogy megfelelő minőségű embriót hozzanak létre. Ez a technika biztosítja a klónozott, vagy genetikailag módosított sertések előállítását, ezzel megalapozza az állattenyésztés, a szaporodásbiológia általános fejlődését, de sok tekintetében a biológiai folyamatok tanulmányozását is. Első ízben kb. 10 éve sikerült laboratóriumi viszonyok között érlelt és termékenyített 2–4 sejtes állapotig fejődött embrió beültetésével malacokat produkálni. Csak röviddel ezelőtt sikerült azonban blasztociszta stádiumban lévő embriók sikeres beültetése. Ez az eredmény lehetővé teszi egy új, fejlett IVP rendszer kidolgozását. Az előadásban bemutatásra kerül a szerző és munkatársai által továbbfejlesztett IVP módszerrel előállított sertésembriók fejlődési képessége és az új technika hasznosítási lehetőségei a biotechnológiában.

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ULTRASOUND GUIDED OVUM PICK UP (OPU) IN CATTLE AND BUFFALO IN ITALY

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INTRODUCTION

Since the first report of the possibility of freezing and thawing semen without loosing its viability (*Polge et al.,* 1949; *Polge,* 1951), and the possibility of extending its lifespan and increasing the conception rate by adding antimicrobial agents (*Foote and Bratton,* 1950), artificial insemination has become the method, used world-wide, to traditionally increase the genetic gain in livestock species through the paternal lineage. This is, to date, still the most efficient way to enhance the genetic merit when using semen of genetically superior males. In large ruminants the maternal contribution to the genetic yearly gain is limited by the physiological barrier constituted by the pregnancy duration and the single resulting offspring. To further enhance the genetic progression, MOET programs have been devised and employed, in which superior cows can be superovulated and fertilized by using superior semen (*Elsden et al.,* 1978). From such elite donors the horns can be non-surgically flushed and the developing preimplantation embryos recovered and transferred into synchronized recipients. This procedure enables donor cows to be free from long lasting pregnancies and be ready to go through the same protocol for additional superior embryo production. Although, when using MOET protocols, a larger number of embryos can be produced resulting in live calves, the maternal contribution is still characterized by a limitation primarily in the number of superovulation protocols to undergo in the course of a year and by an intrinsic possible refractoriness to the hormones used to induce multiple ovulations (*Armstrong,* 1993). The same holds true for buffalo cows (*Bubalus bubalis*), and actually, compared to cattle,

there is a significant reduced results when MOET programs are applied in this species (*Zicarelli et al*, 1993; *Misra*, 1997; *Zicarelli*, 1997a).

According to recent *FAO statistics* (1999), in the last 25 years there has been a 50% increase in the world buffalo population corresponding to a 200% increase in milk production. In Italy, according to some historians, the first buffaloes were introduced as early as the VI century A.D. and no additional introductions thereafter into the Country have been recorded. Furthermore there hasn't been any introduction of germplasm from other Countries where river type buffaloes are bred. This is why recently this species bred in Italy has been identified as "Mediterranean Italian Buffalo". Contrary to all very early predictions, in the last 50 years there has been a dramatic increase in the number of buffaloes, reaching today over 200,000 heads. Most buffaloes are located in 3 regions of Italy, namely Campania, Lazio and Puglia accounting for 95% of the national consistency. Since 1980 though, more buffaloes have been introduced into the northern regions of Italy due to the farming shift from cattle to buffaloes caused by the limitation in cattle milk production for each European Country established by the EEC regulation. In Italy there is a peculiar bond between this species and its most typical milk-derived product, i.e. mozzarella cheese and actually in this Country buffaloes are identified with the idea of mozzarella cheese itself. Very recently, farmers have been attempting to introduce the idea to potential consumers that buffalo meat is also a very healthy and tasty product. Traditionally only few male calves from the best producers are kept in the farm and all the others killed immediately after delivery because of no interest for the benefit of the farmer. A number of studies on buffalo meat though has revealed surprising characteristics regarding its composition (*Gigli et al.*, 2001; *Infascelli and Campanile*, 2001; *Tonhati et al.*, 2001) suggesting the possibility that it may gain part of the beef market arena and the consumer preference if adequately advertised. For this purpose some farmers have already gathered into cooperatives for buffalo beef production after establishing common guidelines for nutrition requirements and general management.

The buffalo species is characterized by a more or less generalized seasonal reproductive performance, usually more pronounced in adult buffaloes compared to heifers (*Esposito et al.*, 1992), variable estrus cycle length, low estrus sign expression or silent estrus and low mounting activity among cows, variable rate of double ovulations and variable interval between estrus and ovulation (*Zicarelli*, 1997ab). The use of artificial insemination is marginal in buffalo breeding due to the low pregnancy rates achieved when synchronization protocols are employed (*Baruselli et al.*, 1997; *Zicarelli et al.*, 1997), and natural mounting is then common practice among farms. Buffalo bulls tend to be replaced when they are 4 to 5 years old due to an intrinsic built up aggressiveness within the herd and against human beings. This contrasts with cattle breeding where, on the contrary, the superior genetic of the same bull can be spread out into the population for many years. Furthermore in Italy the reproductive performances of buffaloes tend to be optimised in the period of the year when more milk is requested for mozzarella cheese production. Unfortunately this contrasts the natural tendency of the buffalo species to have the highest reproductive activity in those months of the year when diurnal light is diminishing (end summer until the end of winter). This is in fact the time of the year

where the highest pregnancy rates can be achieved when using both artificial insemination and natural mounting. On the contrary, to have most of pregnancies by the beginning of spring and summer, when more milk and consequently mozzarella cheese are requested, buffaloes have to be bred at the end of winter/beginning of spring. For this purpose buffalo breeders tend to deseasonalised animals by removing the bulls from the herd in October and reintroducing them in march. It is clear then that most of the genetic progression in this species has been achieved through the maternal lineage, by keeping the most productive buffalo cows (massal selection) and their bull calves within the herd, and capitalizing on their long lasting productivity (most buffaloes are kept in the herd for 10 years or more!).

In vivo and in vitro embryo production in cattle and buffaloes

The highest rate of genetic progression can be achieved through the utilization of the best semen (A.I.) in conjunction with the best milk producers (MOET) according to available genetic indexes and genealogical books for each cattle breed (*Lohuis*, 1995). In cattle such enhancement is made even more efficient by the possibility of trading internationally both semen and embryos. The year 2001 has been funestated by major outbreaks, namely Bovine Spongiform Encephalopathy (BSE) and Foot and Mouth Disease (FMD), which have been responsible for a contraction in embryo production at least in Europe. In Italy preliminary data reveal an overall increase in embryo production for the year 2002 (*Galli et al.*, 2003). In the context of embryo production statistics then, the year 2000 should be considered more representative: by employing MOET programs, over 600,000 embryos have been produced worldwide of which more than 500,000 have been transferred; in Italy, where cattle milk population under productivity control is estimated around 1,300,000 heads among breeds (AIL, 2001), there has been an upward trend in *in vivo* embryo production with more than 12,000 embryos transferred in the same year (*Thibier*, 2001). When taking into consideration *in vitro* embryo production, this usually refers to embryos produced from slaughtered donors as mass production of beef embryos, genetic recovery from valuable donors and Ovum Pick Up (OPU) donors. In the year 2000 there has been a worldwide *in vitro* production of 140,000 embryos of which over 40,000 have been transferred. In Europe in the same year Italy was the Country where more *in vitro* derived embryos (over 6,000) have been frozen and transferred (*Galli et al.*, 2003). The use of OPU in valuable donors for commercial application and *in vitro* embryo production in Italy has become a reality and a tool for genetic improvement since 1996 reaching a peak in 1999 with 2457 embryos frozen for commercial use (*Galli et al.*, 2003).

In the buffalo species, the widespread use of MOET programs is hindered by a more pronounced refractoriness of buffalo cows to ovarian hormonal stimulation and a less efficient synchronization protocols applied to recipients (*Schallenberger et al.*, 1990; *Singla et al.*, 1996). Hormonal stimulation protocols and used hormones are basically the same as employed in cattle because the reproductive physiology of the two species has traditionally been considered very similar. It is likely though that buffaloes may require a modified hormonal stimu-

lation approach and that even some anatomical differences may account at least for part of the unsuccessful attempts (*Baruselli et al.,* 2000). To date, despite the number of investigators involved and experimental approaches tested in the buffalo species, there hasn't been any real progression in the mean number of transferable embryos produced by hormonal stimulation and non surgical embryo recovery (*Singh et al.,* 2000). In Italy at present, despite some early attempts to produce *in vivo* embryos through superovulation, a commercial application and exploitation of such programs in the buffalo species is not viable and as such not applied. Since most of the in-farm genetic progression is based on female massal selection, the use of OPU in the buffalo can be considered an important tool for genetic improvement and is thus more competitive than in cattle when compared to superovulation protocols. Likewise in cattle, the same OPU operative approach is applied to the buffalo species. It is difficult to gain data from Countries around the world regarding the number of buffaloes subjected to OPU sessions for *in vitro* embryo production and research or commercial applications. Over 90% of the total world buffalo population resides in Asiatic Countries (swamp type) and despite global efforts it is possible that some requested informations are not received. Some Countries, like China, have strong programs on genetic improvement of their local buffalo breeds through the implementation of both *in vivo* and *in vitro* embryo production procedures, together with cross-breeding local buffaloes with river type buffaloes (*Nili Ravi and Murrah*) for improving milk production yield (*Xiao,* 1988). In Italy, some laboratories have been engaged in buffalo *in vitro* embryo production by OPU (*Boni et al.,* 1996; *Galli et al.,* 2001; *Neglia et al.,* 2003), although we are still far from a true application of this approach on a large commercial scale. In fact, to date, only few calves have set foot on the ground as a result of OPU and *in vitro* embryo production procedures (*Galli et al.,* 2001).

OPU standard procedure

The OPU technique is a safe, reliable and repeatable procedure that enables the operator to retrieve immature oocytes through the ultrasound-guided puncture of antral follicles. The recovered oocytes will then be processed in the laboratory where they finally will be placed into maturation medium for successive *in vitro* fertilization. A number of ultrasound units are available in the market and most of them have good quality image resolution needed for visualizing antral follicles as small as 2 mm in diameter. Usually a 5 MHz convex array transducer housed in a hard plastic vaginal probe with stainless steel needle guide is used; the frequency of the transducer though can be as high as 6.5 MHz. The gauge and length of the single-lumen needle used for puncturing is also variable being 20G to 17G and 4 cm to 60 cm respectively. Prior to the beginning of the OPU session the animals can be mildly sedated or usually are given an epidural block in order to facilitate handling of the ovaries through the rectum and their puncturing. A vacuum pump is needed, usually with a pneumatic foot pedal and connected to the aspiration line whose length will determine how to regulate the vacuum power in order to have a flow rate between 20 to 40 mL/min. Faster flow rates will yield oocytes with reduced layers of granulosa cells or completely denuded. The aspiration system in place, from the tip of

the needle to the other end of the flushing line into the collection container, is fiiled with heparinised PBS-PVA to avoid clotting in the course of aspiration. Depending on the expected final volume of the aspirated follicular fluid, usually a 15 or 50 mL Falcon tube is used. In order to standardize results, test tubes should be kept into specifically designed heaters at constant temperature of 37 to 38 °C. Size of punctured antral follicles range from 2 mm up to preovulatory diameters. Recovery rate of retrieved oocytes can be very variable, ranging from 50 to 100% of the punctured antral follicles. The setting of the aspiration system will account for the rate of retrieved oocytes, being usually higher with faster flow rate but at the same time yielding more denuded or damaged oocytes.

OPU donors

Domestic large ruminants can be used for OPU through insertion of an intravaginal probe starting at 5 months of age. Smaller and thinner probes can be used for such young prepuberal animals. A similar large number of antral follicles are available few months after birth in bovine and buffalo calves (*Presicce,* in print), contrasting with the difference in total number of germ cells in the ovaries of the two species being significantly lower in buffaloes (*Danell,* 1987; *Van Ty et al.,* 1989;). There are conflicting reports on the need of exogenous gonadotrophins in order to obtain viable competent oocytes for successive fertilization and embryo development in prepuberal calves (*Armstrong et al.,* 1994). It has been shown though that as puberty is approached, there is a an increasing competency to development of retrieved immature oocytes (*Presicce,* 1997). One of the milestones through the *in vitro* production of viable embryos is their maturation into complex media. To overcome this step outside the natural environment being represented by the antral follicle milieu, *Presicce et al.* (2002) have administered gonadotrophins to prepuberal buffalo calves with the intent to obtain matured oocyted at the time of follicular puncture. A significant higher yield of mature oocytes at aspiration, morphologically represented by expanded cumulus cells, has been achieved compared to control calves, reaching an overall 80% of maturation rate when including also retrieved compact COCs undergoing *in vitro* maturation. Aduit females are eligible for OPU under various physiological and pathological conditions: 1) in the course of regular cycles; 2) during pregnancy up to the third or fourth month, after which ovaries are not reached with ease; 3) when not responding to superovulation protocols; 4) to genetically save valuable animals before slaughter, as in the case of forced slaughter in the evidence of epidemiological emergencies; 5) infertile donors under ordinary reproductive management programs. Actually some infertile donors can benefit from ovarian follicle aspiration, especially in case of ovarian cysts, and usually they can come into heat within a couple of weeks from the last OPU session and can be inseminated successfully (*Galli et al.,* 2001). From the data and experience of a number of authors and commercial teams, a twice-weekly OPU session is considered to give the best quality and highest oocyte number (*Tavares et al.,* 1997). In such a schedule, in fact, the first aspiration removes the dominant follicle exerting its influence on the cohort of subordinates present on the ovary and from the second OPU session, only growing

follicles with equal possibility of reaching dominance are available and thus oocyte quality rate is higher and more homogeneous. Likewise in cattle, in the buffaloes OPU can be performed for a long time without altering the future reproductive efficiency. In a recent study by *Neglia et al.* (2003) mixed parity buffaloes were subjected to repeated twice-weekly OPU for 6 months from march to September. This is a period characteristically considered unfavourable for this species at our latitudes. Together with a physiological individual variability in terms of recovered oocytes and embryo development, a reduced cleavage rate compared to cattle oocytes was observed. On the contrary, blastocyst rate from the total number of cleaved oocytes was not significantly different from cattle oocytes, suggesting a generalized higher sensitivity of buffalo oocytes to environmental stress. In the summer months the authors reported a generalized drop off in oocyte recovery and consequently embryo production and this may suggests, compared to cattle, a more pronounced physiological limit to the exploitation of the germ plasm reservoir in buffalo ovaries. Such limit would be set by a combined anatomical reduced number of ovarian follicles (*Danell,* 1987; *Van Ty et al.,* 1989), and by a reduced reproductive efficiency in the period of the year characterized by increasing day length (*Zicarelli,* 1997b).

In vitro embryo production procedures

Details of *in vitro* embryo production procedures are beyond the scope of this manuscript and can be found in other relevant papers (*Hasler,* 1998). Such procedures apply equally to both bovine and buffalo species, and it is important though that an illustration of the basic steps involved is given. Immediately after retrieval, cumulus-oocyte-complexes (COCs) are brought to the laboratory for initial processing. Temperature of the container containing Falcon tubes and aspirated follicular fluid should be kept as constant as possible (37 to 38 °C). On arrival at the laboratory, retrieved follicular fluid is passed through an Em-Con filter and washed several times with warmed up PBS-PVA in order to clear the fluid for better oocyte search. From the filter then, the last wash is moved into a large Petri dish where the first search for COCs is performed. Those found are moved into smaller Petri dish for additional washing until a last wash with maturation medium. From here they are moved into droplets of maturation medium, microdrops or four-well dishes, depending on the system used in different laboratories. Such media are usually Tissue Culture Medium 199 (TCM 199) and Ham's F-10 supplemented with calf serum and hormones (*Singh et al.,* 1989; *Totey et al,* 1993). In buffaloes, although some authors have reported different optimal maturation times (*Kamonpatana and Chuangsoongneon,* 1994; *Neglia et al.,* 2001), usually maturation is carried out for 22 to 24 h in CO_2 incubators at 39 °C. It has been shown that glutathione protects mammalian cells from oxidative stress, and addition of cysteamine, a small thiol compound, to the maturation medium increase glutathione synthesis and the number of embryos reaching the blastocyst stage in cattle and sheep (*De Matos et al.,* 1995). The utilization of cysteamine in the maturation medium in the buffalo species, whose oocytes are characterized by a higher lipid content compared to cattle and thus more vulnerable to oxidative stress has been proved to be beneficial in terms of higher morula and blastocyst rates (*Gasparrini et al.,* 2000). When

OPU is performed in farms very far from the laboratory some portable incubators are used and maturation is started during transportation to the laboratory.

For *in vitro* fertilization, bull semen is usually pre-tested using COCs from slaughterhouse ovaries for determining the best spermatozoa and heparin concentration (*Parrish et al.,* 1988). The fertilization medium as well as the supplementation of other constituents and gas atmosphere may account for significant differences in terms of blastocyst output and normalcy of born calves (*Hasler,* 2000). Tyrode Albumin Lactate Pyruvate (TALP) and Synthetic Oviduct Fluid (SOF) work equally well, although it seems that the latter with the addition of essential and non essential amino acids in 5% CO_2 and 5% O_2 gives a higher output of freezable blastocysts (*Galli et al.,* 2001). To maximize the recovery of the semen motile fraction, a Percoll gradient or a swim up procedure is employed.

Culture of presumptive zygotes represents the last stage of the *in vitro* procedure before transfer or freezing of the developing preimplantation embryos. A number of culture strategies have been adopted over the past years giving variable results. Most laboratories today tend to use defined or semi-defined systems avoiding the need for co-culture feeder cells. In some laboratories, temporary recipients are used yielding usually better results in terms of freezable blastocysts whose rate is not dissimilar from *in vivo* produced embryos (*Galli et al.,* 2001). From a single donor recovered oocytes and subsequently produced preimplantation stage embryos vary between OPU sessions. A number of studies have dealt with various combinations of different numbers of oocytes/embryos and different volumes of culture droplets. Under such conditions it seems that in a droplet volume of 20 µL the number of embryos cultured does not affect development progression (*Galli et al.,* 2001). Development of *in vivo* buffalo preimplantation embryos has been reported faster ($\cong$24 hours) in reaching blastocyst stage compared to cattle (*Drost and Lsden,* 1985; *Karaivanov et al.,* 1987; *Chantaraprateep et al.,* 1989). This physiological aspect can help in identifying the most viable blastocysts produced *in vitro* as those developing faster.

CONCLUSIONS

The combined use of semen from genetically superior bulls and oocytes from the best female producers through OPU can enhance dramatically the genetic progression in both cattle and buffaloes. Both OPU and *in vitro* related laboratory procedures for embryo production have to be optimised in order to maximize results and reduce costs. Contrary to more traditional programs like MOET, OPU-IVEP rely on higher initial costs and specialized laboratories and equipment together with peculiar skills, both inside the laboratory and in the field. In Italy this combined approach for genetic improvement can be applied successfully to both species. In cattle the commercial implementation of this strategy can be, similarly to other Countries, affected by epidemiological contingencies and global marketing. In the buffalo species this approach can be even more interesting and competitive, as genetic progression has always been based on the identification of the best female producers and selection of their bull calves. In the buffalo though, a number of basic aspects related to their

peculiar reproductive physiology still have to be fully understood both in the male and in the female. Such improved understanding will prove beneficial for higher reproductive and productive performances.

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SUMMARY

The traditional genetic improvement through the paternal lineage and bull progeny testing has been strengthened by the application of new reproductive technologies to the female side by means of superovulation protocols and non surgical embryo recovery and transfer (MOET). Some major limitations in the exploitation of the maternal side though have surfaced since the beginning, namely the unpredictable response of suitable donors to hormonal administration and the limited number of MOET programs applicable to each donor. Since the advent of ultrasonography and its application to animal science research and for commercial services, a new strategy to increase the genetic gain in large domestic ruminants has emerged. The parallel refinement of *in vitro* embryo production procedures (IVEP) and ultrasound guided retrieval of cumulus-enclosed-complexes (COCs) has made possible the use of superior females as oocyte donors for successive maturation, fertilization and embryo culture. In cattle this combined technology is already part of the services offered from private companies to breeders together with more traditional reproductive approach. In the buffalo species, characterized by a different herd management and by some physiological peculiarities, this approach is still in its infancy at least in terms of commercial utilization. In Italy an intense activity is registered in the commercial arena of cattle *in vitro* embryo production from superior donors through Ovum Pick Up (OPU), whereas mostly research activity is registered in this field in the buffalo species with some isolated cases of services offered to breeders. In the near future it is foreseeable that also in this more rustic species we will witness a larger utilization of these new reproductive technologies for higher reproductive and productive performances.

ULTRAHANGGAL IRÁNYÍTOTT PETESEJT-KINYERÉS SZARVASMARHÁBÓL ÉS BIVALYBÓL OLASZORSZÁGBAN

PRESICCE, GIORGIO A.

ÖSSZEFOGLALÁS

A tenyészbikák ivadékvizsgálatára alapozott genetikai progressziót, jól kiegészítik az új szaporodás-biotechnológiai eljárásokra alapozott módszerek (tehenek szuperovulációja és az embrió transzfer) és lehetővé teszik a nőivarú állományok tesztelését is (MOET program = Multiply Ovulation and Embryo Transfer). A program széles körű bevezetésével kiderült a nőivar néhány olyan tulajdonsága (pl. a kiválasztott donorok kiszámíthatatlan endokrin reakciója a szuperovulációs hormon-kezelésre, csekély számú érett petesejt keletkezése, stb.), ami eddig ismeretlen volt. Az ultrahangos vizsgálati módszerek kidolgozása és bevezetése a szuperovuláció ellenőrzésére, és a petesejt kinyerésére, új lehetőséget teremtett a biotechnológiai kutatásokban és az embriókereskedelemben. Az ultrahangos készülékkel ellenőrzött érett petesejt-kinyerés vákuumos szonda segítségével és egyéb technikák alkalmazásával megsokszorozta az egy állatból nyerhető petesejtek mennyiségét (cumulus-enclosed-complexes) és javította a kimosott embriók kultiválásának hatékonyságát, továbbá fokozta az *in vitro* termékenyítés eredményességét. Az említett technika ma már részét képezi azoknak a szolgáltatásoknak, amit a mindennapi tenyésztési gyakorlat igénybe vehet A bivalytenyésztésben, a tej biológiai sajátosságai mellett, az említett eljárások még gyermekcipőben járnak, legalábbis, ami a módszer szolgáltatói jellegű biztosítását illeti. Olaszországban a szarvasmarha embrió- és petesejt-kereskedelem széles körben elterjedt, a bivaly fajban azonban ez a módszer még laboratóriumi viszonyok között folyik. Remélhetően a közeli jövőben, ebben a sokkal hagyományosabb fajtában, az új biotechnikai módszerek szélesebb körben elterjednek a nagyobb reprodukció és termelés érdekében.

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MILK LIPID COMPOSITION AND ITS ALTERATION BY ANIMAL BREEDING

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The composition of milk varies greatly among species, and the ratio of fat to protein is quite different among species. This paper will consider only the bovine species and within the bovine the more productive breeds of cattle. As an example, milk from the Holstein breed in the United States contains about 3.4% fat; 3.3% protein; 4.9% lactose; 0.68% ash; and 12.3% total solids. There are many things that influence milk composition in cattle that are both genetic and environmental. Some of these are feed, weather, composition of diet, season of the year, stage of lactation, breed, genetic differences within breeds, and a host of other influences cause milk composition to vary. Genetic factors are the primary source of interest here. Within breed genetic variation is important for the major milk components.

Little is known about the genetics of lactose, even though it comprises the largest solids component of milk. Further, the correlations among lactose and other components of milk have received little emphasis. We investigated variability in lactose when data were available from the Michigan Dairy Herd Association where a part of the cow population was tested for lactose and somatic cell score (SCS) in addition to the usual testing for fat and protein. Somatic cell score is computed from somatic cell counts $SCS=[\log_2 (SCC/100)+3]$, where SCC is somatic cell count. SCS ranges from 1 to 10. Since only a part of the cows were tested for lactose, we compared those data with and without lactose and SCS. The means and variance of the data were almost identical, providing evidence that the data with and without SCS and lactose could be considered as a representative sample from the total cow population, *Welper and Freeman* (1989).

Means and variances: The smallest data analysed for Holsteins was 6,654 first records from cows sired by 266 sires and the largest was 78,163 first records from cows sired by 1364 sires. The smaller data were from cows that had lactose and somatic cell score data. The means for lactose percentage were 4.86 for Jersey cows, 4.84 for Brown Swiss and 4.97 for Holstein cows. The phenotypic and genetic variances were the largest for kg of lactose, followed by fat and protein. For percentage composition, fat had the largest variance followed by lactose and protein (*Welper and Freeman,* 1989). This clearly shows how much variation there is in lactose.

Heritabilities and correlations: Table 1. shows the heritabilities, genetic and phenotypic correlations among milk, its components and somatic cell score (SCS). The heritabilities estimates of milk, fat, protein, and lactose yields are 0.30, 0.28, 0.28, 0.26, respectively. For percentage composition the heritabilities for fat protein and lactose are 0.41, 0.46, 0.43, respectively. These values are consistent with literature estimates. The heritability for SCS is estimated to be 0.16. Changes in these traits can be made rather easily if selection is applied. However, the correlated traits must also be considered, *Welper and Freeman* (1991, 1992).

Table 1.

Genetic correlations (above diagonal), heritabilities (on diagonal) and phenotypic correlations below the diagonal among milk and its' composition

	Milk	Fat	Protein	Lactose	Fat, %	Protein, %	Lactose, %	SCS
Milk	**0.30**	0.71	0.93	0.92	−0.40	−0.47	−0.30	0.15
Fat	0.81	**0.28**	0.79	0.68	0.35	−0.04	−0.16	0.12
Protein	0.96	0.85	**0.28**	0.89	−0.21	−0.12	−0.21	0.18
Lactose	0.96	0.79	0.94	**0.26**	−0.35	−0.37	0.10	0.13
% fat	−0.34	0.27	−0.20	−0.30	**0.41**	0.59	0.16	−0.06
% protein	−0.36	−0.06	−0.09	−0.27	0.51	**0.46**	0.29	0.01
% lactose	−0.08	−0.02	0.01	0.20	0.11	0.29	**0.43**	−0.11
SCS	−0.04	−0.07	−0.04	−0.08	−0.06	−0.01	−0.15	**0.16**

The genetic correlations among kg of milk and kg of the fat, protein, and lactose traits are high and positive indicating selection for one trait can be expected to also change other traits. The genetic correlations among milk, fat, protein, and lactose and percentage composition are smaller and mostly negative, except between kg of fat and fat percentage. Genetic correlations among percentages are positive, but not as large as among kg of the traits. Phenotypic correlations follow the same general pattern as the genetic ones but tend to be lower. The correlations with SCS are all positive except those for percent fat and percent lactose. Note that a positive correlation is detrimental, meaning that selection to lower SCS would decrease yield of milk and kg of the other components.

Expected change in milk composition by selection: Knowing the heritabilities and correlations as presented in *Table 1.* allows predicting genetic response over time. We developed a program that simulates 2-stage selection in dairy cattle with parameters that are representative of the Holstein population in

the United States. It allows for different selection differentials for buli mothers, young sires to be progeny tested, and among progeny tested sires, different generation intervals, and different genetic and phenotypic parameters and economic weights for the traits. The program when applied to some artificial insemination (AI) organizations in the United States has predicted change rather accurately.

Table 2. shows predicted genetic changes based on the values in Table 1. and for the relative economic weights that are specified in Table 2. Table 2. gives predicted change based on difference economic weights, Welper (1991). Twenty years of selection was simulated. Somatic Cell Score is included as a measure of mastitis. The last row of Table 2. gives the expected response for single trait selection for only the trait in that column. For example, single trait selection for only milk would be expected to produce 2846 kg in 20 years. When an economic weight of 4 is applied to both milk and protein, the response is 2714 for milk, 75 fat, 81 for protein, 121 for lactose. Responses are negative for percentages of the traits with an increase of .30 for SCS. The latter is a large undesirable response that would have a negative effect on udder health, reduced production, and income because premiums are paid for milk with low SCS.

Table 2.

Expected responses from 20 years of multiple trait selection for milk composition

Milk	Fat	Protein	Lactose	Fat	Protein	Lactose	SCS
kg				Percentage			
2714 (4)*	75	81 (4)	121	−0.23	−0.08	−0.17	0.30
2708 (2)	75	80 (8)	121	−0.22	−0.08	−0.17	0.18 (−1)
2725 (2)	75	80 (8)	115	−0.23	−0.09	−0.24 (−1)	0.31
2723 (2)	80 (1)	80 (4	121	−0.17	−0.09	−0.18	0.30
2562	87 (3)	78 (6)	108	−0.03	−0.06	−0.22 (−1)	0.16 (−1)
2436	80 (1)	71 (2)	92	−0.05	−0.08	−0.31 (−1)	−0.09 (−1)
2846**	98	81	135	0.87	0.39	−0.64	−1.35

*: Economic weights in parenthesis   **: Direct response to single trait selection

There are some generalities that can be made from Table 2. Selection for yield will generally reduce percentage concentration of the components. The converse is selection for increased concentrations will decrease kg (not shown). The correlation structure is strong enough that when effective selection is applied to one or more traits the other traits will change even though no direct selection is applied to them. All relative economic weights increase SCS except when small positive weights are given to fat and protein and negative weights to percentage lactose and SCS, then SCS is expected to decrease by −0.09. If selection was for only SCS, it would be expected to decrease −1.35 units. This would be accompanied by reductions in yields. This can be seen where a weight of one was given to fat, two to protein, and a minus one to lactose and SCS, the SCS was decreased by −0.09. Unless SCS has a very large economic value, too much emphasis on SCS will reduce income more than is gained by selection on this indirect measure of mastitis. Mastitis is important to the health of the cow and in many places in the United States a bonus is paid for low SCS

because using milk with low scores results in retail products of higher quality. Some small direct or indirect selection against mastitis might be justified; however, good management can control mastitis.

Differences in fatty acids in normal milk: Causes of variations in fatty acid content of milk have been shown to be differences between stage of lactation, date of sampling, lactations, herd, season of calving, breed, diet of the cow, and genetic differences among cows, when the genetic effects have been corrected for environmental differences. Two of the earlier studies (*Kajarijord et al.,* 1982; *Syrstad et al.,* 1982) used milk from 30 daughters sired by 114 test bulls. They estimated heritabilities for the fatty acids from C6:0 to C18:3. These estimates were between 0.05 and 0.26, with most close to 0.15. Genetic correlations were generally positive with milk, fat and protein. A notable exception was the correlation with C15:0, which was negative for all three-production traits. This work indicated that selection could change the fatty acid composition of milk. *Kajarijord et al.* (1993) found that the proportion of most short chain fatty acids were positively, and the proportions of most long chain fatty acids are negatively correlated with fat content when computed across cows. The opposite relationship was found within lactations.

Syrstad et al. (1982) estimated repeatabilities and found very low repeatabilities for C18:1, C18:2, and C18:3. They also found that the change in fat percentage during a lactation can be attributed to the long chain fatty acids, particularly C18:0 and C18:1. They suggested that this could be due to the feeding conditions in Norway. Their estimated genetic correlations were very large among the C:6 through C:14 fatty acids.

Milk products from milk with unsaturated fatty acids: Work with fatty acids at Iowa State was initially motivated to develop a method of breaking the genetic correlation between fat and protein. During the course of this work, it was discovered that there was natural variation between cows in the fatty acids in their milk when fed the same diet, *Beitz et al.* (2000). Because saturated fats have been implicated in human health, work was continued investigating the variability in fatty acids. *Ubricht and Southgate* (1991) developed an index of athrogenicity defined as the ratio IA=(%12:0+4(%14:0)+%16:0)/sum % unsaturated fatty acids. Based on repeated sampling of about 400 Holstein cows in two herds the IA index varied from 1.24 in the low IA cows to 3.70 for the high IA cows. The properties of the milk for manufacturing products were examined from high and low IA index cows by making several products. Two trials were conducted making butter. Butter from both trials was firmer from the high IA cows than the low IA cows and butter from the low IA cows was less firm spread like margarine and tasted like normal butter. Further, swiss and cheddar cheese had little change in texture and again tasted like normal cheeses. Ice cream followed the same pattern. Cows could not be permanently classified as high or low IA because the IA index changes with stage of lactation and age of the cows. Generally, cows at the beginning of first parity had the lowest IA and old cows near the end of lactation had the highest IA. A near infrared procedure is being developed that can separate high and low IA cows on a real time basis.

Association of fatty acids and milk proteins: To compare milk fatty acid composition of cows with different milk protein phenotypes, 592 milk samples from 233 Holstein cows were analysed for κ-casein and β-lactoglobulin phenotypes and fatty acid composition, *Bobe* (1997). Cows with κ-casein BB genotypes had higher concentrations of laurate and lower concentrations of stearate in milk fat than did cows κ-casein AB and AA , whereas cows with κ-casein AA had higher concentrations of palimitate in milk fat than did cows with κ-casein AB. Cows with β-lactoglobulin BB had higher concentrations of myristate, palimitate and palmitoleate and lower concentrations of stearate and linoleate in milk fat than did cows with β-lactoglobulin AA, and cows with β-lactoglobulin AB had intermediate values. The results suggest that cows with different κ-casein and β-lactoglobulin phenotypes differ in their milk fatty acid composition with higher concentrations of de-nova synthesized fatty acids at the expense of stearate in cows with κ-casein BB and β-lactoglobulin BB, respectively.

Phenotypic correlations among individual proteins and fatty acids were computed from 233 Holstein cows that were representative of the US Holstein population based on similarity of blood types (*Bobe et al.,* 1999). The phenotypic correlations among the quantities of six major protein fractions were all high, and ranged from 0.62 to 0.95. Also, the phenotypic correlations among the 11 fatty acids were large and positive. The correlations among the fatty acids and the protein fractions ranged from 0.56 to 0.83, with most being between 0.6 and 0.75. The relationships among the individual milk proteins and fatty acids suggests a possible pleitrophic effect. However, a search of the data base in the National Center for Biological Information showed the enzymes that are involved in synthesizing fatty acids and the individual proteins are on different chromosomes.

To determine the association among individual proteins and fatty acids a factor analysis was performed. Total phenotypic correlations were used in the analyses (*Bobe et al.,* 1999). The first grouping from the factor analysis included C6:0 through C16:0; the second grouping included all of the protein fractions; third was three fatty acids C16:1, C18:1 and C18:2; fourth was C4:0 and C6:0; the fifth included C4:0 and C6:0. Factor 6 and 7 had high loadings on C18:0, β-lactoglobulin and C16:0. From these analyses it is clear that there is an association among the fatty acids and the individual protein fractions in milk. A review of the composition of bovine lipids, *Jensen* (2002) gives an extensive review primarily covering the biochemical aspects of lipids, but does not contain any detailed genetic information.

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SUMMARY

The ability to genetically change the total fat and fat percentage is well known. More recently total protein has been studied and breeders have demonstrated the ability to change total protein. Total fat and protein have a well-defined correlated structure, and heritabilities that are in the moderate to high range. Continuous effective selection for only production will reduce fitness as demonstrated by the undesirable relationship with somatic cell score. Changing the fatty acids in milk has not received much interest from animal breeders. Heritability estimates of individual fatty acids from separate populations are generally in the moderate to high category indicating that selection to change any fatty acid would be effective, but correlated changes in other fatty acids would result. Changes that could occur may not be beneficial to human health. It has been demonstrated that dairy butter, cheese, and ice cream can be made from milk with lower saturated fats that have normal manufacturing properties and tastes like products made from normal milk.

A TEJ LIPID ÖSSZETEVŐI ÉS MÓDOSÍTÁSUK TENYÉSZTÉSI ELJÁRÁSSAL

FREEMAN, A.E.

ÖSSZEFOGLALÁS

Ismeretes, hogy a tejzsír mennyisége és %-os aránya genetikailag megváltoztatható. Az utóbbi időben a kutatók figyelme táplálkozás-élettani jelentősége miatt, főleg a fehérjetartalomra irányult. A tejzsírnak és fehérjének jól meghatározott összefüggő szerkezete van, és öröklölhetősége a közepes és a magas között változik. A tej beltartalmi értékei széles variációban változnak, a tehén fajtája, típusa, a takarmányozás módszere, a laktáció stádiuma, stb. függvényében. A tejmennyiségre irányuló szelekció közismerten csökkenti a szaporaságot, romlik az állatok egészségi állapota és kedvezőtlenül alakul a tej szomatikus sejtszáma. A tenyésztők ma még nem tanúsítanak kellő figyelmet a tejzsír zsírsav-összetételére és annak az ember számára történő kedvező irányú megváltoztatására. A tejzsír zsírsav-összetétele, különböző populációkból származó tejben meglehetősen változó, ami a tenyésztési módszerekkel történő megváltoztatás lehetőségeire utal. A zsírsav-összetétel jól öröklődő tulajdonság, tehát a szelekció hagyományos eszközeivel is módosítható. A tej zsírsav-összetételére irányuló szelekció, azonban a többi zsírsav-komponensben bekövetkező esetleg nemkívánatos változáshoz vezethetne. Ez kedvezőtlen hatást gyakorolhatna az ember egészségére. Ismeretes, hogy megfelelő tej és tejtermék készíthető több, nagyobb mennyiségű telítetlen zsírsavat tartalmazó tejből is megfelelő tejipari gyártástechnológiával, és az íze is megegyezik a „normál" tejből előállított készítményével.

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FOOD SAFETY AND QUALITY TRAITS FOR MEAT AND MEAT PRODUCTS:
NATIONAL AND INTERNATIONAL IMPACTS

PUOLANNE, EERO

INTRODUCTION

In terms of quality, there are three categories of realities: "real reality, RR" (i.e. the actual concrete items, meat and meat products), "numerical reality, NR" (numbers characterising the material or samples of it), and finally the "psycho reality, PR" (the mentai response of a person to the stimulus of senses, modified by his/her mind).

Which one of these can be the home site of The Quality? There is no absolute definition or unit for (meat) quality. From the point of view of producers and manufacturers the first two categories are much utilised and are also rather exact and satisfactory measures. The weight and number of items (RRs), fat content, rancidity, microbial load, measured colour, even results of sensory tests (NRs) etc. are useful traits of fitness for use and further on for the economical value of the products. Many times, an exact distinction between carcass quality and meat quality is not made. Carcass quality is a product of animal breeding and animal husbandry as well as perimortal treatments. Meat quality is also a product of those, but it can be altered by certain measures, such as cutting, sorting, ageing etc.

More or less for the consumer quality is a psychological term (PR), which means that the product must, in the form of sensory reactions, pass the "semipermeable" barrier of the mind to create the impression of quality. Naturally, there are many aspects of quality that can be expressed as numerical etc. information, as for instance the residue content, fat and salt contents, microbial load, method of preparation, ingredients other than meat and additives etc. Although these to a certain extent provide absolute values, there still remains a psychological factor in them. The psychological response is mostly built on pre-

vious experiences with meat consumption, e.g. aroma, satisfaction resulted from the structure and satisfied feelings. It is not known to what extent the response is based on our instinctive reactions. It is assumed that our reactions are more or less of a general nature, i.e. desire for salt, fat, protein (?) etc., and not so much dealing with quality aspects. The psychological reactions to various foods are strongly related to ethnic and socio-economical factors as well as e.g. religion, not to mention individual variation based on physiological and experience-based factors.

For consumers, and hopefully also for producers and the meat industry, the ethical aspects are important. Ethical concerns are related to animal welfare, the treatment of meat animals and the efficiency of muscle food production and its environmental impacts. Ethics is a relevant factor regulating our psychological reactions towards meat and meat products and, by doing so, influencing "the psychological" taste, aroma, nutritive value, production and environmental efficiency. It has been clearly seen that when a consumer sees one problem in this context, let it be fat content, the treatment of animals, or pollution caused by animal production etc., all of these aspects are rapidly fused to create a wholistic negative attitude against meat.

Meat safety is an important part of meat quality. Short-term safety concerns microbial load, especially the numbers (RR) or counts (NHR) of pathogenic bacteria. Animal diseases and zoonoses are parts of microbial safety, also from a consumer point of view. Chemical residues and contaminants are more or less long-term aspects of health concerns. Finally, to a certain extent, the chemical macro and micro composition of meat can be a safety issue from the nutritional (or toxicological) point of view.

As discussed concerning quality in general, safety has a psychological dimension: BSE, foot and mouth disease, pathogenic avian influenza, heavy metals, etc. are health concerns, but these concerns also influence the psychological quality of items that are absolutely free from those agents. In regards to ethics and meat safety, the psychological quality is dichotomic ("yes/no"), not comparative like most other aspects of quality ("this sausage is better than that"). A consumer is not usually analysing the quality, and consequently it is expressed on a one-dimensional scale like "this is better", and hardly it is said "this tastes better, but it is tougher", and never as the following: "this is more tender, but the relative risk of CJD is significantly higher".

Therefore, "quality" is not only the quality of the product itself, but also the quality of the mode of action.

This review shortly covers the central aspects of meat and meat product quality and safety. Because of the general title of the review, given to the authors, no references are given. A special focus will be on the internationalisation of meat trade and eventual effects of the need to increase the productivity on animal welfare and meat quality. The authors emphasise the benefits of efficient animal production to provide inexpensive and wholesome meat with the lowest possible environmental impact. It is, however, important to evaluate the eventual consequences on animal welfare and meat quality.

Meat safety

Chemical: The chemical safety of meat and meat products has markedly improved during the last few years. The residues and contaminants are controlled much more efficiently than before, especially due to EU and USDA regulations. EU strives for a totally hormone free meat, but sporadic positive findings indicate that some illegal use of hormones still exists. The Trans-Atlantic battle about the use of hormones is still alive, and recently it was again shown that hormones do not cause a risk for consumers. It may be that hormones do not cause a market risk for consumers, but we do not have proof that animal welfare is not endangered. The use of growth hormones increases the growth rate, which may mean a delay in the development of supporting systems and connective tissues. Anabolic hormones increase selectively the growth of muscular tissues, resulting in an increased live weight of the animal relative to the "normal" live weight and strength of connective tissues at the same age. An increased incidence of bone defects could be expected, as seen with an increased growth rate, even without added hormones. The muscle tissues generated by anabolic hormones have a decreased oxidative capacity due to the lower capillarisation of the muscles.

Toxic residues and contaminants have a dual development. Simultaneously, when less toxic chemicals are used in industrial fields, there is a need for more efficient production and higher total quantities of food to fulfil the increased needs of the growing population, while more soil, and more polluted soil will be utilised for food production. Also, the lack of high quality water will be a limiting resource for safe and sufficient food production in larger areas of the world.

There are some contaminants in meat processing that raise health concerns. Polycyclic aromatic hydrocarbons (PAHs) will be produced in higher amounts in traditional smoking processes. Their amounts must be reduced by controlling the smoke formation (pyrolysis) temperature to below 450 °C or 650 °C, and the energy for product cooking must not be from the same wood material from where the smoke is generated. In the modern meat industry there are separate smoke generators and steam generators, but in small-scale operations (and also for some fermented products) the traditional way of production results in elevated PAH contents over the commonly used limit of 1 microgram/kg expressed as 3,4-benzpyren. PAHs are also produced when fat is in contact with hot surfaces. Especially harmful is grilling, which is a very common method of preparation for meat and meat products. Another group of contaminants that are less known, are the process-induced mutagenic compounds. They are pyrolysis products of meat constituents, meat proteins in general, and especially creatine.

Microbiological: World statistics show that food infections and intoxications are frequent, but the situation is improving in developed countries. When the lengths of food chains from field to fork increase and the sizes of food operations increase, the control of the chain is in many cases easier than before, but the risk of larger epidemics is still present. Several pathogenic microbes have found their new niches in modern production chains. A typical example is *Escherichia coli* O157:H7, which is very difficult to eradicate from the production

chain. The only practical destruction method is to cook the product thoroughly to temperatures over 70 °C to destroy *E. coli* (e.g. 72 °C/2 min, 80 °C/6 sec.). It should also be noted that the bacteria can survive in pH 4 for a longer period of time. Therefore, there is no simple method to eliminate the strain in fermented sausage except simply avoiding having it present in the raw materials.

Also, nitrite is still an issue in meat products. In Finland, where small children like mild cooked sausages, the acceptable daily intake -value (ADI) for children is almost 90%, and in a quarter of children it is exceeded. Therefore, the EU upper limit 150 mg/kg $NaNO_2$ is much too high. A recommendation for significantly lower values, 70–80 mg/kg level, can be proposed. However, the risk of *C. botulinum* that can grow at low nitrite levels is strongly stressed.

Microbial safety is most efficiently guaranteed by good handling practices and packaging of the products. The elimination of microbial contamination at processing may result in products of high keepability. One possible new risk is that when total contamination is reduced, pathogenic bacteria that are accidentally introduced may have improved possibilities to grow. Therefore, proper packaging (and eventually modified atmosphere packaging) are needed for a safe product. Low temperatures are most essential for safety, although some pathogenic bacteria, e.g. listeria are able to survive/grow in very low temperatures, even below 0 °C.

Nutritional:

Fat: Meat fats are usually mentioned as a major risk factor for coronary heart diseases. The foundations of these claims are usually not very solid. The fat content of meats has been reduced markedly during the last few decades. The driving forces have been the recommendation of human nutritionists to avoid animal fats and to increase of the efficiency of feed conversion. The fatty acid composition of the major meat species are rather close to the widely accepted recommendations, e.g. the oleic acid content of most meat fats are over 40%. Naturally, the harmful palmitic acid content (about 25%) is high and the contents of polyunsaturated fatty acids are rather low, but the very critical voices against meats are not particularly justified, especially in countries where the share of meat fats is lower than 25% of total fats.

There is an alarming increase in obesity and diabetes in developed countries. There are some indications that diets with high soluble carbohydrate content (high clycemic load) are a factor that increases this tendency. High glycemic load results in the accumulation of fat around the waste area (so called "apple-type" fatness). If this hypothesis would be proven to be true in the near future, it will mean that meat, which is rich in protein and sometimes also fats, but low in carbohydrates, would be accepted again by nutritionists.

Salt: When consuming meat products and other muscle foods, the intake of salt (sodium) may comprise a significant risk. In Finland it has been estimated that 10% of the population has elevated blood pressure, and 40% will suffer from that during the later period of their life. Salt has not been an issue in most countries, but e.g. in Finland much attention has been paid to this problem. The salt content of cooked sausages can easily be reduced to a level of 1.4% when phosphate is used, without influencing negatively the technological quality, but below that level the taste of the product decreases markedly and even turns to

a non-typical taste. We have been able to show that it is high contents of meat protein that decreases the perceived saltiness, not fat or other proteins. Therefore, both for technological (water-binding, gel stability) and taste reasons, the very low salt contents can be used in low meat, high added-water sausages.

Meat quality traits

Fresh meat:

Tenderness: Tenderness is a most important quality trait for consumers. The tenderness of meat is especially important in beef and lamb, where the interaction of myofibrillar proteins and connective tissue proper have an influence. The perimortal treatments (stress, cooling) and ageing are important. In pork and poultry, the tenderness is of minor importance, mainly due to the very low contents of connective tissue. Also the post mortem reactions are very fast, especially pH fall, decreasing the importance of the immediate post mortem tenderisation caused by proteolytic enzymes, mainly calpains.

Tenderness is of importance in large meat particles. Products made of minced or chopped meats or containing phosphate are not critical to the issue of tenderness. On the other hand, the low content of connective tissue and high internal temperature utilised to cook pork and poultry basically nullifies the effects of connective tissue, and the tenderness is mainly influenced by the state of the actomyosin complex.

Colour and taste: Due to the increased growth rates of the meat animals the animals are slaughtered at younger ages than before. The fastest growing meat animals (poultry, swine) have white fibres as a large part of their muscle. White fibres grow faster than red and intermediate fibres, at least in early stages of development in the animal. Consequently, meat with a high proportion of white fibres is light in colour. Also the fat content in general and intramuscular fat in particular is low, which results in reduced taste. This reduced taste can also be attributed to the tendency of reducing the salt contents of meat and meat products.

Water-binding: Water-binding is the most important trait of meat. More precisely, water-binding, fat-binding, tenderness, colour, taste, juiciness, and gel-forming ability are more or less derived from the same basic structural factors. The formation of the actomyosin complex and the eventual denaturation of the structural proteins during the post-mortem reaction sequence influence strongly the functional properties of meat. The amounts and properties of connective tissue also influence the properties, especially the tenderness, but also, to some extent, the water-binding and fat-binding abilities. The basic variables influencing also here are the rate of pH fall, perimortem temperature and ultimate pH.

Meat products

Fat, salt and nitrite were discussed above. In meat products these are important quality traits, although they are mainly treated as sensory or microbial safety factors. Additionally, there is a marked increase of ready-to-eat foods taking place in the industrialised countries. The borderlines between the industry and retail shops and restaurants are changing towards the industry, which is

selling its services to further elements of the food production chain. Consumers buy time and know-how; many of them are not able to prepare the food themselves. The particular responsibility of the manufacturers is to optimally design the products nutritionally and to ensure the satisfactory safety of the products. Organoleptic aspects may lead to high-fat and high-salt products. Therefore, levels of fat and food additive contents must be such that an every-day use of the products can be recommended.

International trade and protectionism

Economical aspects

Food production is of paramount economic importance in most countries. It has a close relationship to employment, especially in rural areas, and in many countries food production has a crucial role in export. Taking the two lines in extreme the first (which are related employment) try to reduce the import to protect their own agro-industry, but the other extremes (focusing on exports) try to open all borders to have open export markets. In today's world and especially in EU, it is not allowable to place restrictions on free export/import. Legal barriers are also not allowed, but there are still strong barriers caused by national food habits. Particular food items, their taste and aroma, way of preparation etc. makes it very difficult to cross the border. Raw materials, however, cross the borders with greater ease.

Real safety reasons

Because the safety limits of products are different for different countries, there is an impact on eating and preparation habits. E. g. sausage consumption is higher in some countries than in others, and children do not eat sausage in some countries at all, but do so in others. This renders it important to have different regulations or formulation policies in different countries. In Finland, for instance, we are concerned with the high intake of nitrite and salt of small children, and therefore we have developed rather low salt, low nitrite and low fat sausages.

Another area is pathogenic bacteria. In Finland we have practically eradicated salmonellae from our food chain. This has been achieved by using the competitive exclusion techniques, creating a very efficient control system for broiler slaughtering and processing. Therefore, a very strict control of salmonellae in imported foods is also used.

ACKNOWLEDGEMENT

SUMMARY

The definition of quality is complex, including such factors as the product itself and the modes of action and the perception of quality, influenced by the psychologial reactions of consumers. The control of the chemical hazards of foods has improved, and in developed countries no particular problems exist. Some concerns can be attributed to polycyclic aromatic hydrocarbons and process-induced mutagenic compounds. Also the present intakes of salt (sodium) and nitrite may be too high. The most essential risk is related to total nutrition. The associated nutritional risks caused by various relative intakes of fat, carbohydrates, red meat etc. remains still unresolved. Meat quality is based mostly on fat and connective tissue contents and the post mortem state of the actomyosin complex. This is mostly influenced by carbohydrate metabolism (pH) and time-temperature courses immediately after slaughter.

HÚSOK ÉS HÚSIPARI TERMÉKEK ÉLELMISZER-BIZTONSÁGI ÉS MINŐSÉGI JELLEMZŐI: NEMZETI ÉS NEMZETKÖZI HATÁSOK

PUOLANNE, EERO

ÖSSZEFOGLALÁS

A minőség összetett fogalom, ami magába foglalja magát a terméket, az összes fontos fizikai, kémiai, biológiai, stb. sajátosságaival. A minőség érzékelésével, a termék működésének jellegzetességeivel a vásárló ízlésével, pszichológiai reakcióit váltanak ki. Az élelmiszerek minőségbiztosítása következtében, azok kémiai, bakteriológiai, stb. veszélyei (ártalmai) az ember számára, a fejlett országokban semminemű problémát nem okoznak. Némi aggodalomra adhatnak okot a kondenzált többgyűrűs heterociklusos aromás szénhidrogének (polycyclic aromatic hydrocarbons) és a termelési folyamatokban keletkező, vagy jelenlévő mutagén hatású anyagok. A táplálékban felvett sók és nitrit mennyisége is esetenként túlzott lehet. A leglényegesebb táplálkozási kockázatot a napi szárazanyag-felvétel összetételének és mennyiségének az ingadozása jelenti. A zsírok, szénhidrátok, a vörös húsok, stb. változó relatív felvételéhez kapcsolódó élelmezési kockázatok szerint, a humán táplálkozásban, még mindig megoldatlan gondok jelentkeznek. A hús minőségét — többek között — zsír- és kötőszövet-tartalma és a benne lévő akroniozin vágás utáni (post mortem) állapota határozza meg. Ezt leginkább a szénhidrát anyagcsere (pH) és a vágás utáni idő-hőmérséklet viszonyok befolyásolják.

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RESEARCH AND DEVELOPMENT LESSONS FROM THE FOOT AND MOUTH DISEASE AND BSE OUTBREAKS IN EUROPE

SHANNON, DAVID W. F.

INTRODUCTION

During the last fifteen years the U.K. has been the focus for two major animal disease outbreaks each of which has had important implications for other European countries and internationally. Bovine Spongiform Encepha-lopathy (BSE) was first discovered in cattle in the U.K. in 1986 and subse-quently developed into a major disease outbreak in the U.K. with smaller num-bers of cases in Switzerland and some EU member countries. The disease was given dramatically new importance in 1996 when it was concluded that BSE had given rise to a 'variant' of the fatal human disease, Creutzfeldt-Jakob Dis-ease (vCJD). These diseases are typified as being neurodegenerative, having incubation periods extending into years and being invariably fatal.

The other major epidemic occurred in 2001 and involved Foot and Mouth Disease (FMD). FMD is a highly contagious viral disease affecting cattle, sheep, goats and pigs and to gain control of the U.K. outbreak (referred to as FMD2001) required the slaughter of many millions of sheep and cattle. A series of independent inquiries have subsequently sought to identify lessons for con-tingency planning and eradication policies. The aim of this paper is to focus

more narrowly on lessons for R&D from these very different disease episodes drawing as appropriate on the official reports and the author's personal involvement.

Brief descriptions of the epidemics

BSE and vCJD in the U.K.

In December 1986 a novel cattle neurodegenerative disease was first identified in the U.K. (*Wells et al.*, 1987). It was one of a group of fatal diseases of both animals and man known as the spongiform encephalopathies because of the typical spongy deterioration of the brain. It was subsequently given the name Bovine Spongiform Encephalopathy (BSE). It was later shown to be transmissible experimentally to other animals and hence falls into the group known as the transmissible spongiform encepathologies(TSE's). These diseases typically have incubation periods in terms of years and when the first case of BSE was discovered in the U.K. a significant but unidentifiable epidemic was already in prospect. It was soon discovered that the means of transmission of BSE was the meat and bone meal (MBM) included in cattle feeds. The practical difficulties of completely excluding MBM from ruminant feeds added to the size of the epidemic in the U.K. and allowed it to spread in the EU. The epidemic in cattle peaked in 1992 but small numbers of cases still occur in the EU and a new case was identified in Canada this year emphasising the continuing threat from this disease.

Until 1996, BSE had been found primarily in cattle but in March of that year the U.K. Government announced that BSE had probably been transmitted to humans and thereafter the importance of the disease increased dramatically. The new human disease was classed as a variant of CJD (vCJD) the existing human SE. The incidence of vCJD since 1996 is shown in Figure 1 as a proportion of the other known diseases in this group.

Fig. 1.: **Deaths from definite and probable CJD in the U.K. 1990–2002**
(Data from CJD Surveillance Unit, Edinburgh, May 2003)

□CJD-Totals ▩vCJD
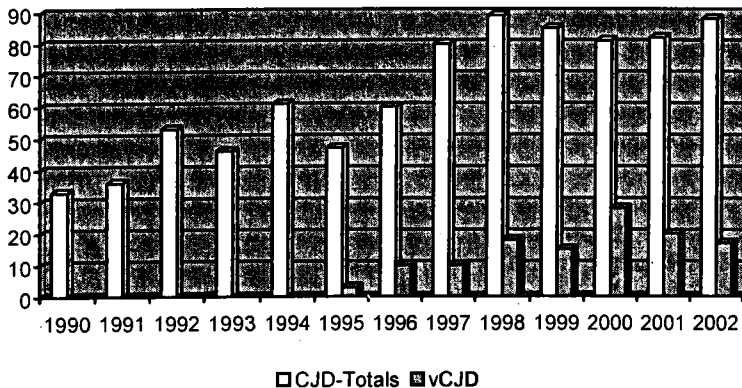
Recent analysis suggests that the incidence may have peaked but the medical implications for surgical procedures and blood transfusions, including the international aspects, have been enormous.

FMD 2001

Between February and September 2001, the U.K. experienced one of the worst epidemics of FMD the modern world has experienced. It was the first major outbreak in the U.K. since 1967–1968.The severity of the epidemic was in considerable measure due to a rare combination of circumstances in which the virus had unknowingly been dispersed to 57 farming premises before the first cases were identified. Interestingly, in this respect it may have simulated a bioterrorist attack rather than a typical outbreak of the disease. It was also predominantly associated with the sheep flock, making diagnosis more difficult. Before the epidemic had been extinguished some 3.5 m sheep and 0.5 m cattle had been slaughtered. These numbers do not include very young animals or those slaughtered for animal welfare reasons. The scale of the epidemic raised many issues surrounding the handling of major acute animal disease epidemics involving slaughter policies, namely the means of disposal of carcases, the potential role of vaccination and the impact on rural industries such as tourism.

Lessons for science from the official Inquiries

The BSE Inquiry

The. U.K. government funded an exhaustive review (The BSE Inquiry, 2000) of all aspects of the BSE epidemic in the U.K. In the scientific area, the recommendations from the review focussed most significantly on the provision of scientific advice. The way scientific advice was sought and used became a major issue affecting trust in Government particularly when, in 1996, the earlier advice that BSE was most unlikely to transmit to humans had to be reversed. The Inquiry recommendations focussed on two aspects of advice namely, the relationship between Government and its scientific advisers and the operation of advisory committees themselves. The Inquiry recommendations are now codified in guidelines for Departments (Guidelines, 2000). Additionally Government was advised to retain 'in-house' sufficient scientific expertise to ensure that departments were able to identify where they needed advice and were able to frame appropriate questions, to understand and critically review the advice given, and to act on it in a sensible and proportionate manner.

Recommendations on research management stressed the importance of effective coordination across government agencies and departments and the use of tendering and peer review to enhance the quality and range of proposals received. The importance of having contingency funding available to meet unexpected needs was stressed, as was the need for plans to make best use of limited specialist expertise.

Finally the report stressed the importance of 'horizon scanning' to help Government identify future risks and research questions that are not being addressed adequately.

FMD Inquiries

A series of independent inquiries (e.g. *Anderson*, 2002) examined the background and handling of the epidemic and the U.K. Government has published its response (Response to The Reports of the Foot and Mouth Disease Inquiries, 2002). Most reports underline the importance of contingency planning to avoid some of the pitfalls that occurred in the U.K. and as part of its response, the U.K. Government published a FMD contingency Plan on its web site. The most relevant in the context of this paper is the review of science carried out by The Royal Society (*The Royal Society*, 2002). It commended the quality of the individual groups in the U.K. but commented that the research effort on FMD was fragmented and not as effective as it could be. They recommended a National Centre for Animal Disease and Surveillance and a National Strategy for Research in Animal Diseases. The expectation was that the Centre would be a 'virtual centre' embracing new university-based research units in specific areas complementary to those within the Institutes. An extra £250 m funding over 10 years was advocated, to strengthen research groups and provide expensive large containment research facilities. Much greater research effort was needed on field epidemiology and modelling to help prepare for an epidemic, to predict its course and to model different control strategies. Similarly diagnostic tests could be improved and vaccines offering lifelong sterile immunity were advocated. Finally they recommended the establishment of a new Priorities Board for research into farming and food matters.

Practical lessons from FMD 2001

The first and perhaps the most obvious practical lesson was that with an acute disease such as FMD, eradication measures must be undertaken on the basis of existing knowledge. Time does not permit significant new research to be undertaken. Long-term studies of virus transmission, potential vaccines or even improved carcase disposal methods could not yield results in time to assist with control of a current outbreak. Studies to support existing control measures such as strain typing the virus and assessing relative infectivity of the virus were carried out but otherwise the aim was to use existing knowledge effectively and to learn as much as possible from this epidemic to assist in the control of future epidemics. In this context analysis of the vast body of epidemiological data collected in FMD 2001 will be most important for the future. Apart from the time constraint the need to redirect key research expertise and facilities to disease eradication tasks left little resource to set up new research. The one major exception was modelling.

The second practical lesson related to the potential role of epidemiological modelling in evaluating control strategies and assessing progress during an outbreak. Some aspects of the modelling in FMD 2001 such as the use of contiguous culling (*Ferguson et al.*, 2001) proved controversial and may have been flawed (*Honhold et al.*, 2003). But the power to investigate alternative control strategies became apparent where reliable data was available. On this occasion the modelling was handicapped by the limitations (for this purpose) of the existing annual census data and the difficulty of feeding back accurate and timely

data from the control operations themselves (*Anderson*, 2002). Nevertheless the current emphasis on traceability in the food chain should ensure much better data for simulation studies of future outbreaks. The vast body of field data collected in FMD 2001 is being prepared and will be made available to bona fide researchers. Hopefully analysis of this data will yield further insights into the spread of the disease and the relative effectiveness of the various control strategies employed.

The third lesson is the difficulty of doing research to address the applied aspects of FMD in countries that are normally free from the disease. The limitations on secure disease facilities means that experiments may take many years to obtain the necessary replication to give statistically valid results. This may explain more is known about the structure of the virus from laboratory-based studies but rather less about the practical aspects of viral spread in the field.

The fourth lesson is the need for much greater co-ordination of research effort and more cooperation and planning between the founders of FMD research. Whilst FMD scientists communicate with each other it was clear during FMD2001 that a number of groups were working on parallel diagnostic tests with limited co-ordination between them. The scale of the research effort needed cannot readily be provided by any single country and the control measures used need to meet internationally agreed standards. This points to a much more integrated international approach in future.

The constant need to challenge emerging assumptions

It is inevitable that at the start of a major disease epidemic there is a need to formulate rationales to help to explain how the epidemic had arisen. The public and the media reasonably demand explanations. Hence in the U.K. a popular view emerged that BSE resulted from feeding large quantities of concentrate feeds, including MBM, to dairy cows. Media commentators and interest groups, who objected to intensive farming systems and wished to use the occurrence of BSE in the U.K. in support of their cause, readily accepted this explanation. But such simplistic explanations can easily lead to mistaken policies. In the case of BSE it was subsequently shown that transmission of the disease occurred in the later stages of calf rearing and that very small quantities of infective brain (less than 0.2 g of dry brain) given by mouth were sufficient to cause disease. The earlier view suggested that policy measures, which substantially reduced the consumption of bovine MBM by cattle, would be sufficient to bring the disease under control. The occurrence of cases beyond that expected when the early control measures were introduced, demonstrated that only the strictest exclusion of infective material (MBM) would control the disease.

Another important fallacy that might have had disastrous consequences was the assumption that mice would provide the best experimental model on which to test tissues for infectivity. Mice had been the main experimental model for the study of scrapie and specific strains of mice were available (in small numbers initially) which together could be used to identify the various strains of scrapie. Mice also had the advantage of being capable of being kept in large numbers under laboratory conditions and hence were used in bioassays to de-

termine which bovine tissues carried infectivity and needed to be excluded from the human diet. However some argued that despite their widespread experimental use, mice were not likely to be the most sensitive species on which to test for infectivity. Mice are not naturally affected by TSE's, perhaps suggesting a higher resistance to this group of diseases. Again a feature of individual TSE's is that they tend to be more infective to the species in which they occur. When the sensitivity of mice and calves to BSE was tested experimentally it was found that mice were more than 1000 times less sensitive than calves. This means that bovine tissues that had been screened as safe for human consumption by mice might still have contained 1000 infective units per g if fed to a calf. Fortunately the evidence from the vCJD data in the U.K. suggests that humans are as insensitive to BSE as mice, otherwise controls on the consumption of bovine products might need to be considerably stricter. These are but two examples of the types of initial assumption that can acquire credibility. The lesson is that all assumptions must be challenged rigorously at the start and continuously as new evidence emerges.

CONCLUSIONS

The two major disease epidemics (BSE and FMD) to strike the U.K. in recent years are described. In different ways these epidemics have had major implications for public health, international trade and for the use of science by Governments to control diseases. The conclusions, for R&D, of the subsequent review reports are summarised and some practical lessons are outlined. The potential role of epidemiological modelling in future outbreaks is emphasised. The international implications of animal disease epidemics such as these and the scale of the research needs identified, demands increased cooperation and coordination. Finally some examples are given to illustrate the need to constantly challenge emerging assumptions and to approach problems with an open mind.

ACKNOWLEDGEMENTS

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SUMMARY

The two major disease epidemics (BSE and FMD) to strike the U.K. in recent years are described. In different ways these epidemics have had major implications for public health, international trade and for the use of science by Governments to control diseases. The conclusions, for R&D, of the subsequent review reports are summarised and some practical lessons are outlined. The potential role of epidemiological modeling in future outbreaks is emphasised. The international implications of animal disease epidemics such as these and the scale of the research needs identified, demands increased cooperation and coordination. Finally some examples are given to illustrate the need to constantly challenge emerging assumptions and to approach problems with an open mind.

KUTATÁSI ÉS FEJLESZTÉSI TANULSÁGOK A SZÁJ- ÉS KÖRÖMFÁJÁS, VALAMINT A BSE JÁRVÁNYBÓL EURÓPÁBAN

SHANNON, DAVID W.F.

ÖSSZEFOGLALÁS

Az előadás, az elmúlt években az Egyesült Királyságot sújtó két fő fertőző betegséggel (BSE és FMD) foglalkozik. A szóban forgó megbetegedések jelentős mértékben befolyásolták a nemzetközi kereskedelmet, a közegészségügyi helyzetet és a különböző kormányoknak, a betegség megfékezésére irányuló kutatási programjait. Az előadás az ebben a témakörben megjelent publikációk és kutatási jelentések legfontosabb megállapításait foglalja össze, és körvonalaz néhány gyakorlati tanulságot. Foglalkozik a járványok kitörésének epidemiológiai modellezésével és ennek potenciális szerepével a betegség leküzdésében, illetve megelőzésében. A hasonló járványos megbetegedések jelentős nemzetközi hatások és kutatási igények miatt nagyobb összefogást és koordinációt tesznek szükségessé. Néhány gyakorlati példa segítségével szemlélteti az előadó, hogy az elképzeléseket folyamatosan felül kell vizsgálni, és az új gondolatokat nyitottabban kell kezelni, már sokoldalúságuk miatt is, a járványügyi szituációkat újszerűen kell megközelíteni.

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