What is the Future of Animal Biotechnology ?

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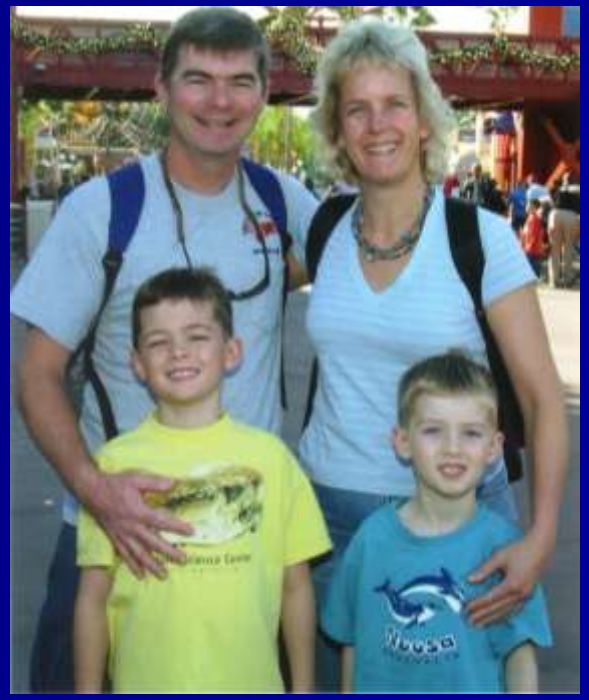
10/17/2006

Animal Genetics 107 (1986)

I[illegible]?



"The public opposes animal biotechnology.."

- The majority (56%) of Americans oppose scientific research into genetic modifications of animals.

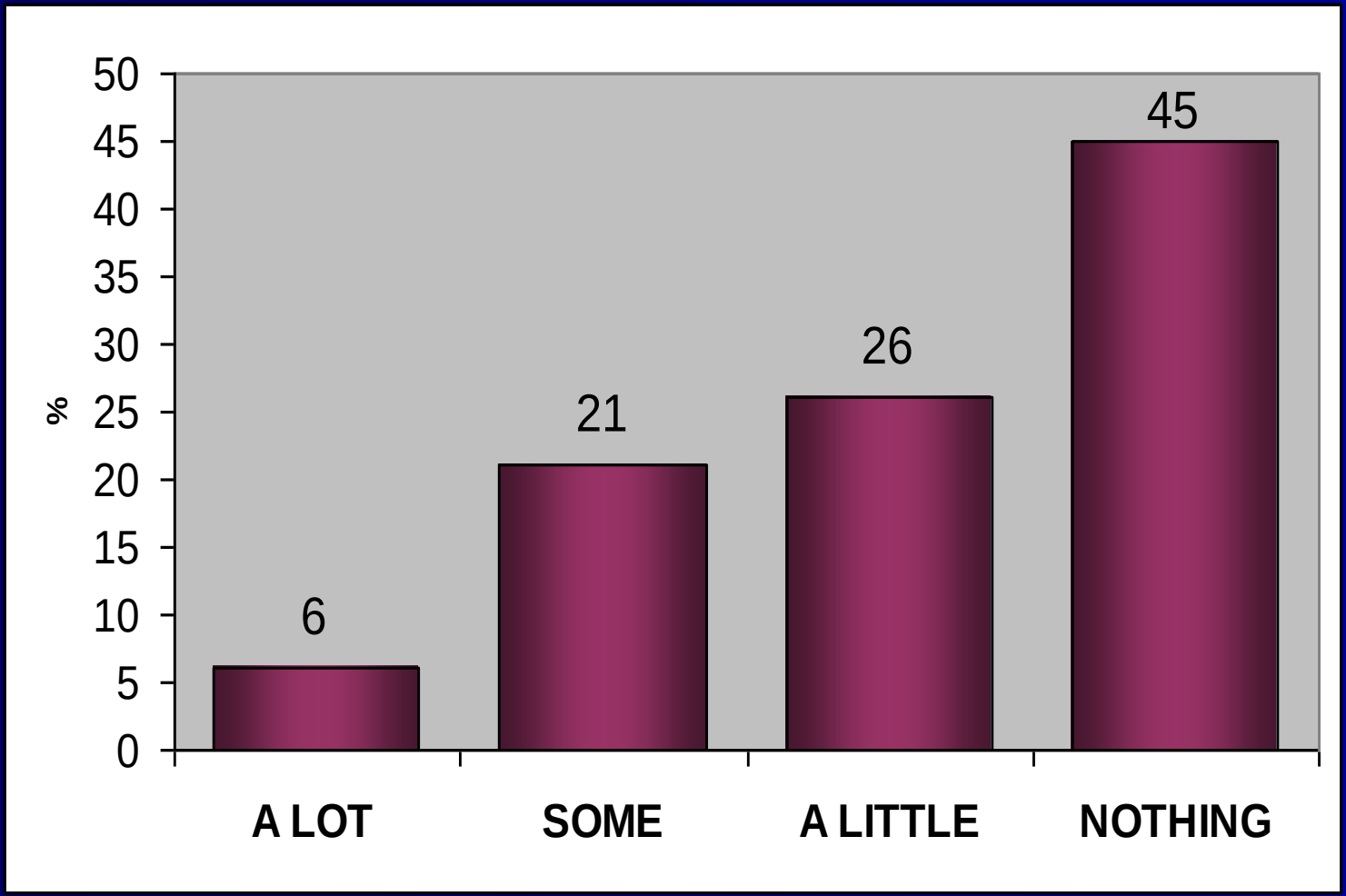
http://pewagbiotech.org/research/2005update/2005summary.pdf

- It should be noted that acceptance of traditional animal crossbreeding techniques was previously found to be only 31 %, with 50% of respondents finding such practices morally wrong

Schilling, B. J., Hallman, W. K., Adelaja, A. O., and Marxen, L. J.2002. *Consumer Knowledge of Food Biotechnology: A Descriptive Study of U. S. Residents*. Food Policy Institute, Cook College, Rutgers - The State University of New Jersey. 25p. http://www.foodpolicyinstitute.org/

US Public Attitude Surveys

How much have you heard about animal biotechnology ? (IFIC, 2005)



http://ific.org/research/upload/2005BiotechSurvey.pdf

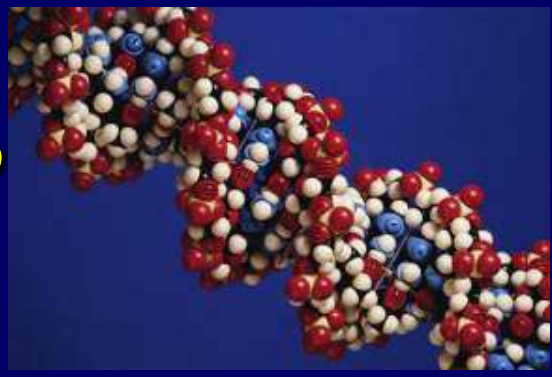
"I know it when I see it"

Of the people who say they know nothing about biotechnology, genetic engineering or genetic modification; almost half (46%) disapprove of the use of genetic modification to create plant-based foods, and 66% disapprove of animal-based genetic modification.

Hallman, W. K., Hebden, W. C., Aquino, H.L., Cuite, C.L. and Lang, J.T. 2003. *Public Perceptions of Genetically Modified Foods: A National Study of American Knowledge and Opinion.* Rutgers - The State University of New Jersey.

Genetically-modified animals

What is Biotechnology ?



Biotechnology

Technology based on biology. The application of science and engineering to living organisms.

What did you hear/read about animal biotechnology ?

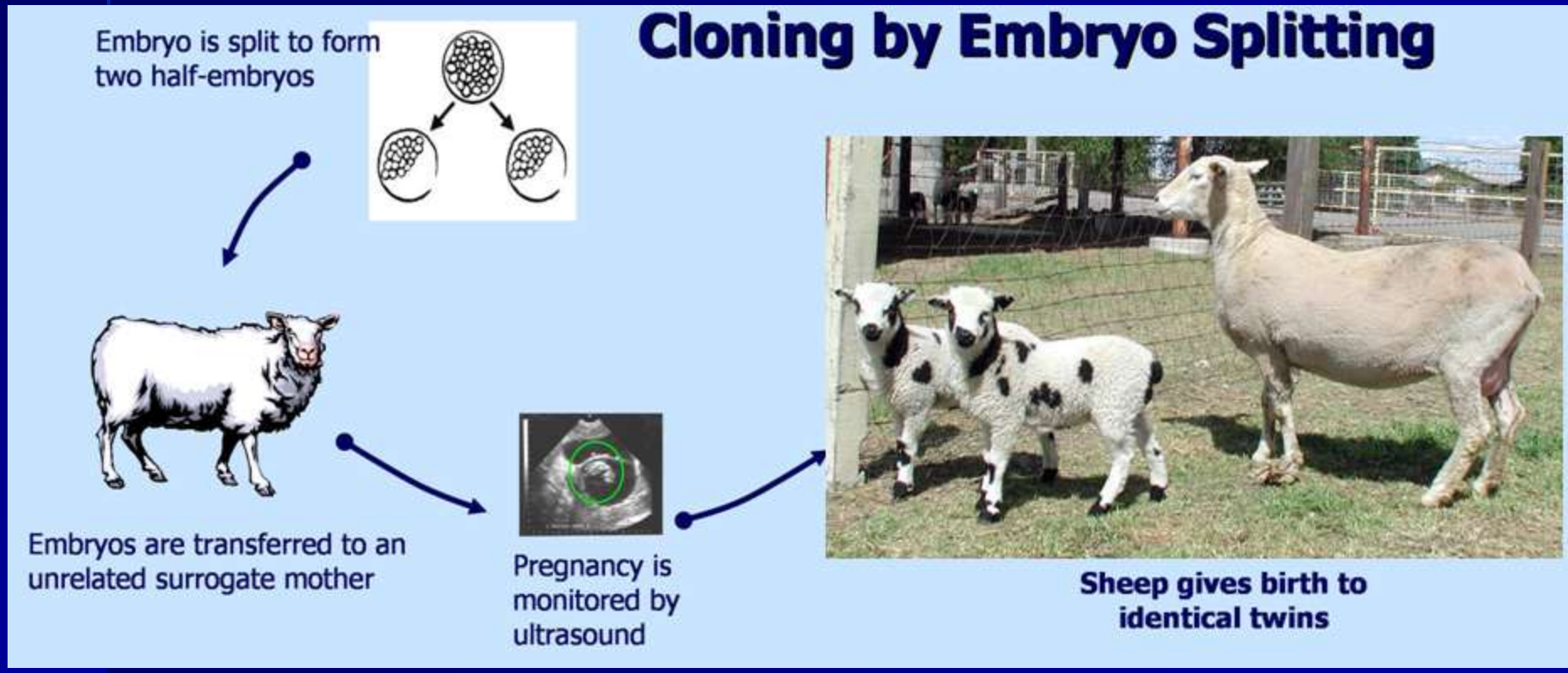
Cloning	15 %
Faster Growth/Bigger Animals/More Meat, Milk, Eggs	11 %
Hormones	10 %
Nothing Specific	13 %
Genetic Engineering	6 %
Disease Resistance/Healthier Animals/Safer Food	5 %
Changes to Animal Feed	4 %
Better Quality Meat	5 %
Concerns about effects/Testing needed	4 %
Steroids	2 %
Testing on Animals/Raised in Labs	4 %
What is Fed to Animals/ How animals Fed	4 %
Breeding/AI	2 %
Mad Cow	3 %
Other	7 %
Don't Know/Refused	27 %

http://ific.org/research/upload/2005BiotechSurvey.pdf

Animal biotechnology

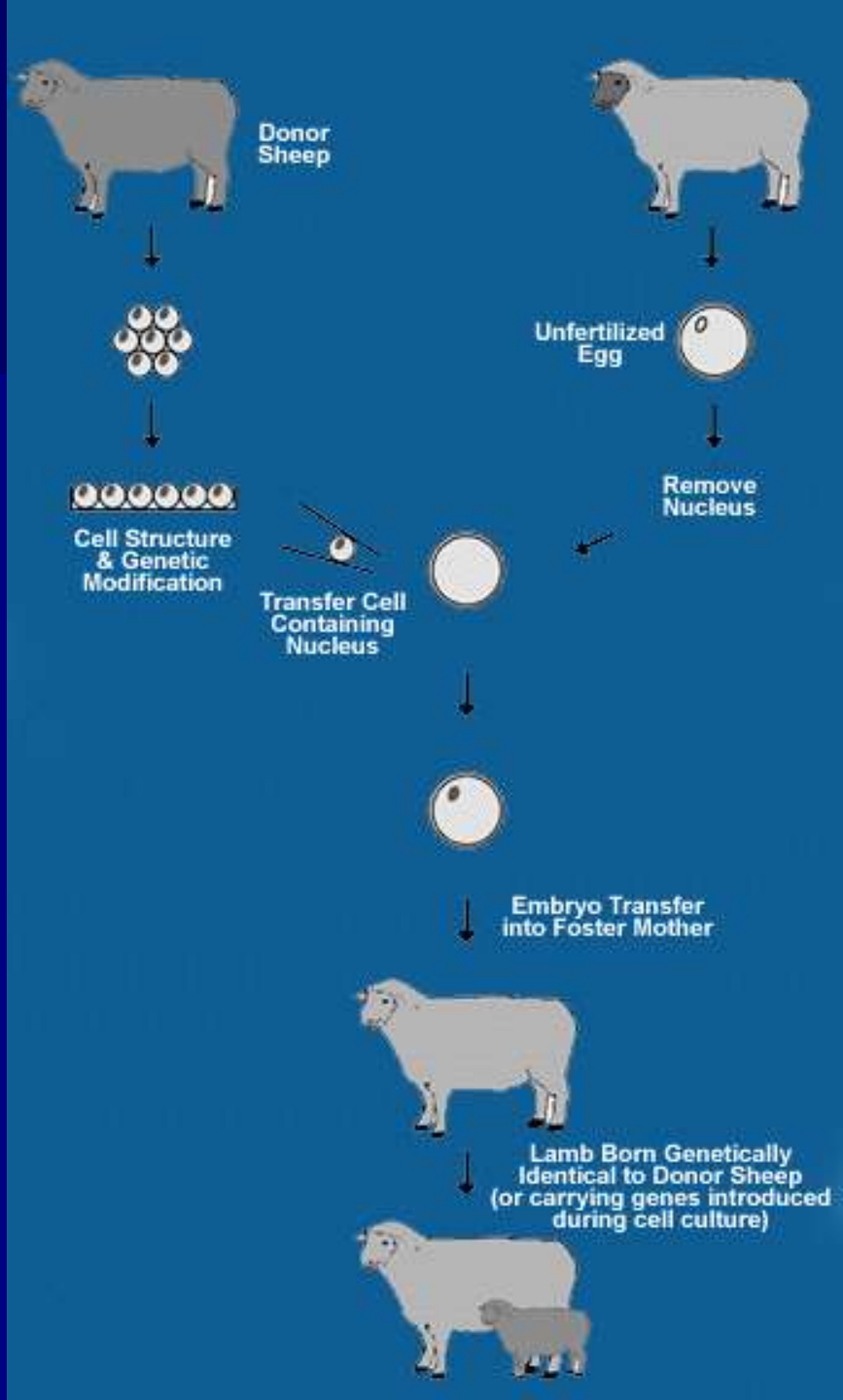
- Artificial selection (breeding programs)
- Artificial Insemination
- Hormone use
- Using DNA information for the marker-assisted selection of superior animals
- Genomics
- Cloning
- Genetic engineering

Cloning by embryo splitting



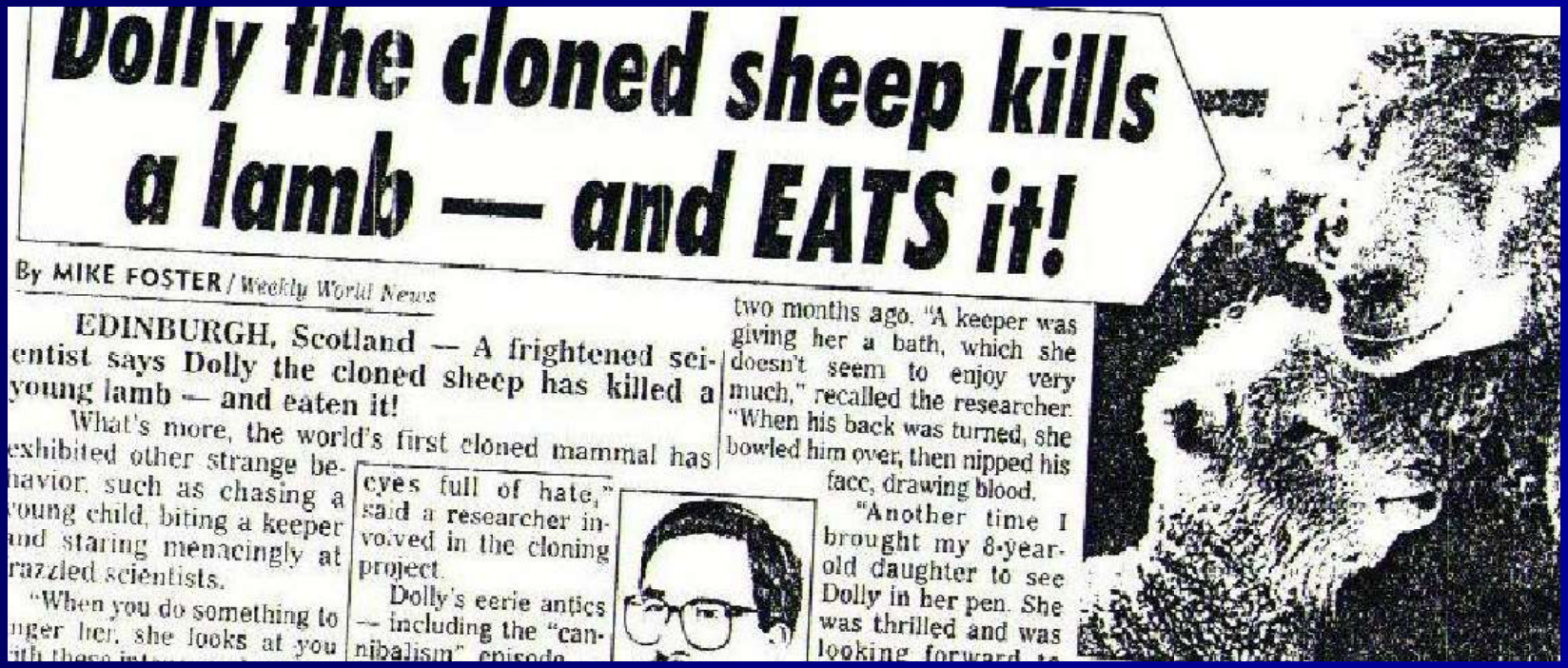
Cloning by embryo splitting

Dolly (1996), the first adult SCNT clone



Dolly rapidly became entangled with the debate over human cloning

Ensuing discussion failed to elaborate on the reasons as to why cloning was developed



Dolly the cloned sheep kills a lamb — and EATS it!

By MIKE FOSTER / *Weekly World News*

EDINBURGH, Scotland — A frightened scientist says Dolly the cloned sheep has killed a young lamb — and eaten it!

What's more, the world's first cloned mammal has exhibited other strange behavior, such as chasing a young child, biting a keeper and staring menacingly at razzled scientists.

"When you do something to nger her, she looks at you ith these int

eyes full of hate," said a researcher involved in the cloning project.

Dolly's eerie antics — including the "cannibalism" episode

two months ago. "A keeper was giving her a bath, which she doesn't seem to enjoy very much," recalled the researcher. "When his back was turned, she bowled him over, then nipped his face, drawing blood.

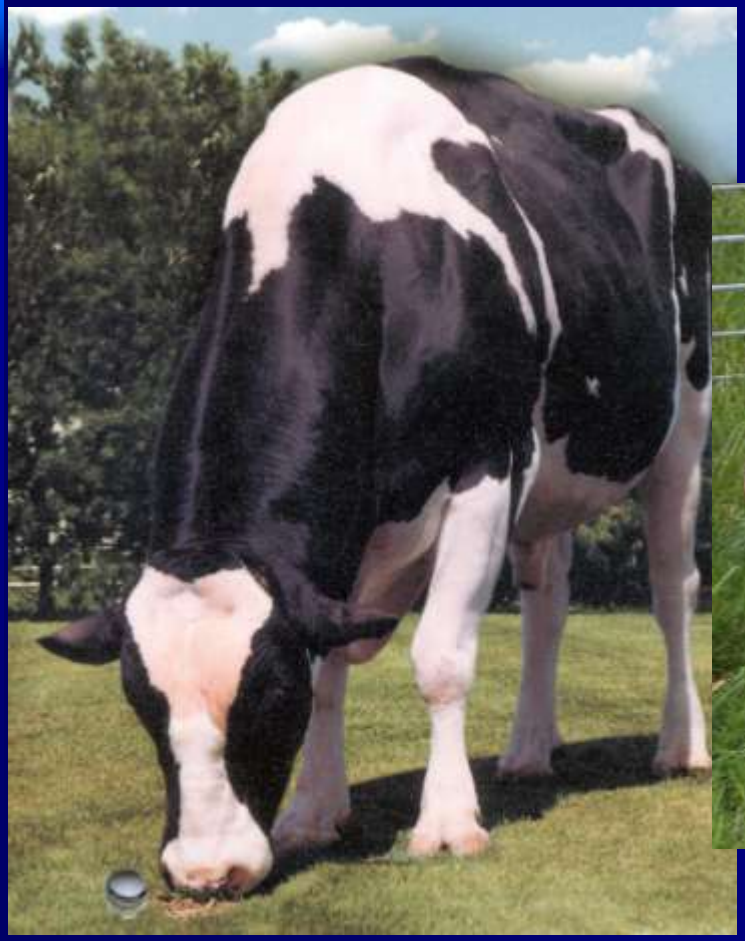
"Another time I brought my 8-year-old daughter to see Dolly in her pen. She was thrilled and was looking forward to

Many animal species have been since been cloned from adult cells

Who's Buying?

$20,000

Regancrest Emory Derry died unexpectedly.



Who's Buying?

- **Full Flush**

Unable to supply market demand for his semen

The FDA continues to call for a voluntary prohibition of the marketing of milk or meat from SCNT clones and their offspring

Are the milk and meat from SCNT clones safe for human consumption?

1. All studies have shown that food products derived from clones fall within normal industry standards or previously reported values for milk and meat.
2. Sample sizes are small in all studies – although there are an increasing number of studies published

FDA ASSESSMENT OF ANIMAL CLONING

"food products derived from animal clones and their offspring are likely to be as safe to eat as food from their non-clone counterparts, based on all the evidence available."

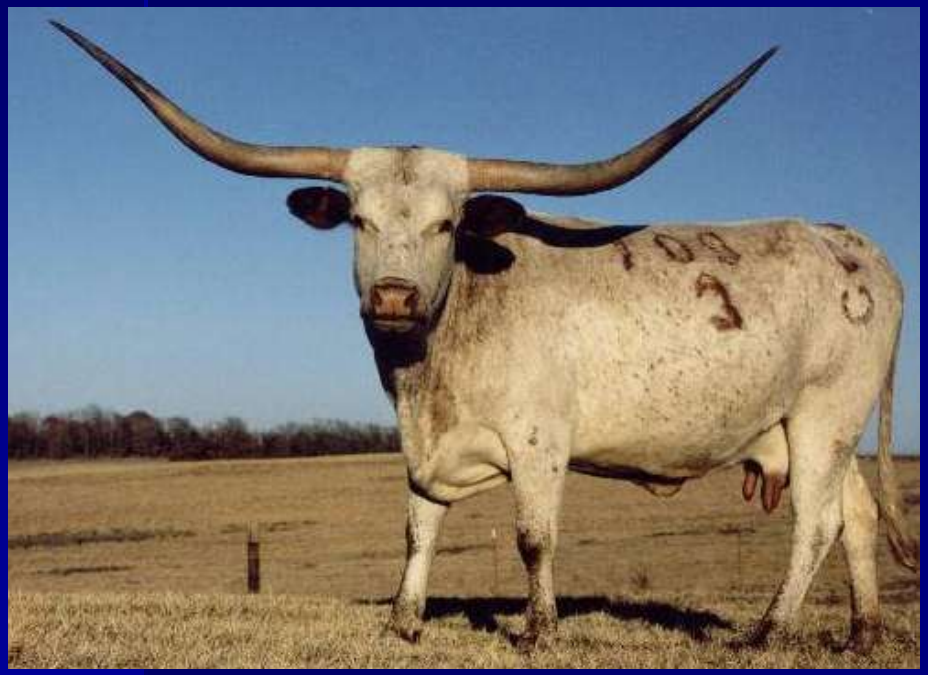
http://www.fda.gov/cvm/Documents/CLRAES.doc (issued 10/31/03)

Milk and meat from cloned animals to market this year?

Who's Buying?

Specialty Cattle Producers

Starlight: **record 77 inches 'tip to tip'**

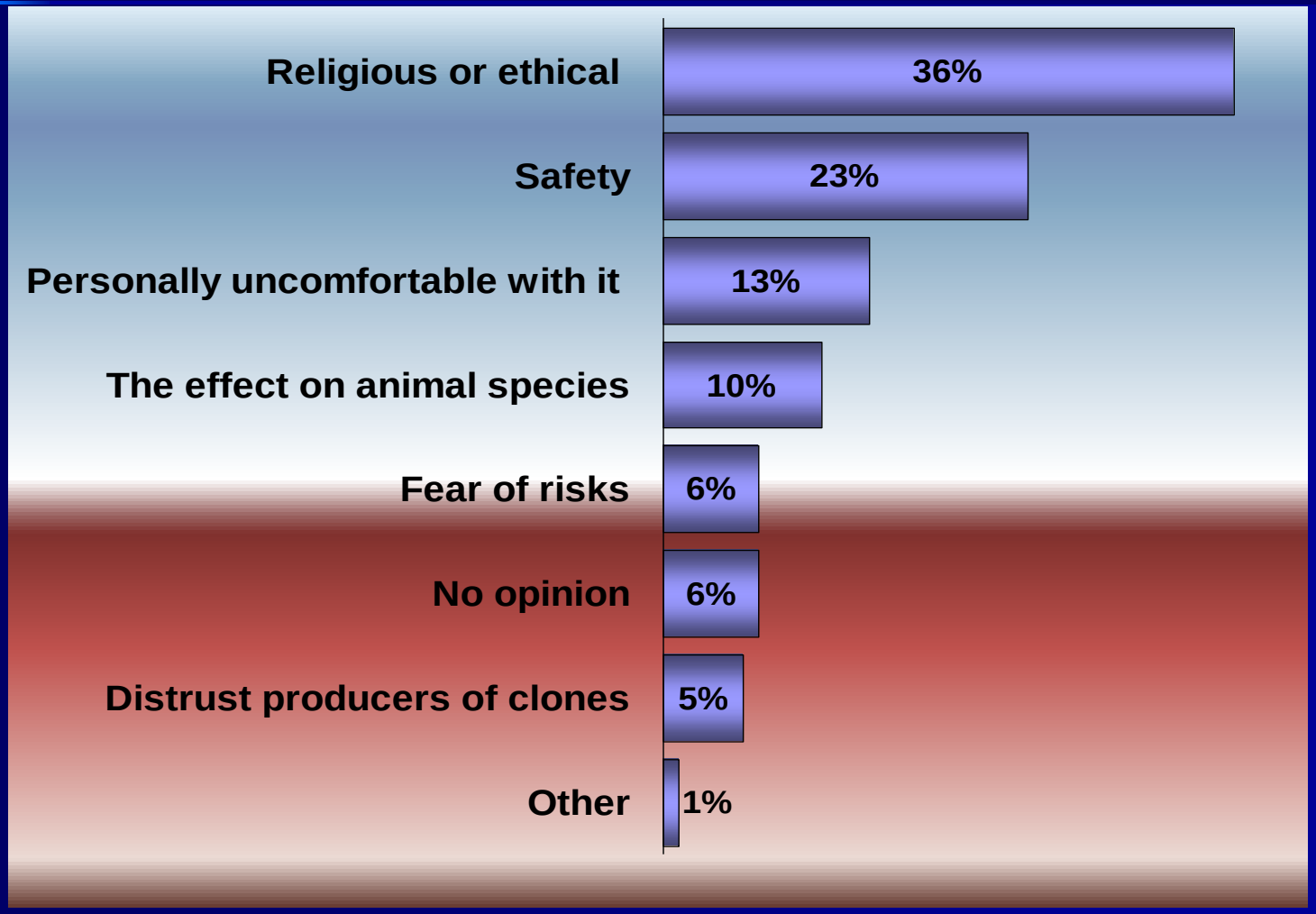


Ethical Issues: Cloned Gizmos and Snuppies

Little Gizmo, clone

http://www.savingsandclone.com/

Of the Americans who are uncomfortable or unsure about animal cloning; their primary concern is:



10/2005

http://pewagbiotech.org/research/2005update/2005summary.pdf

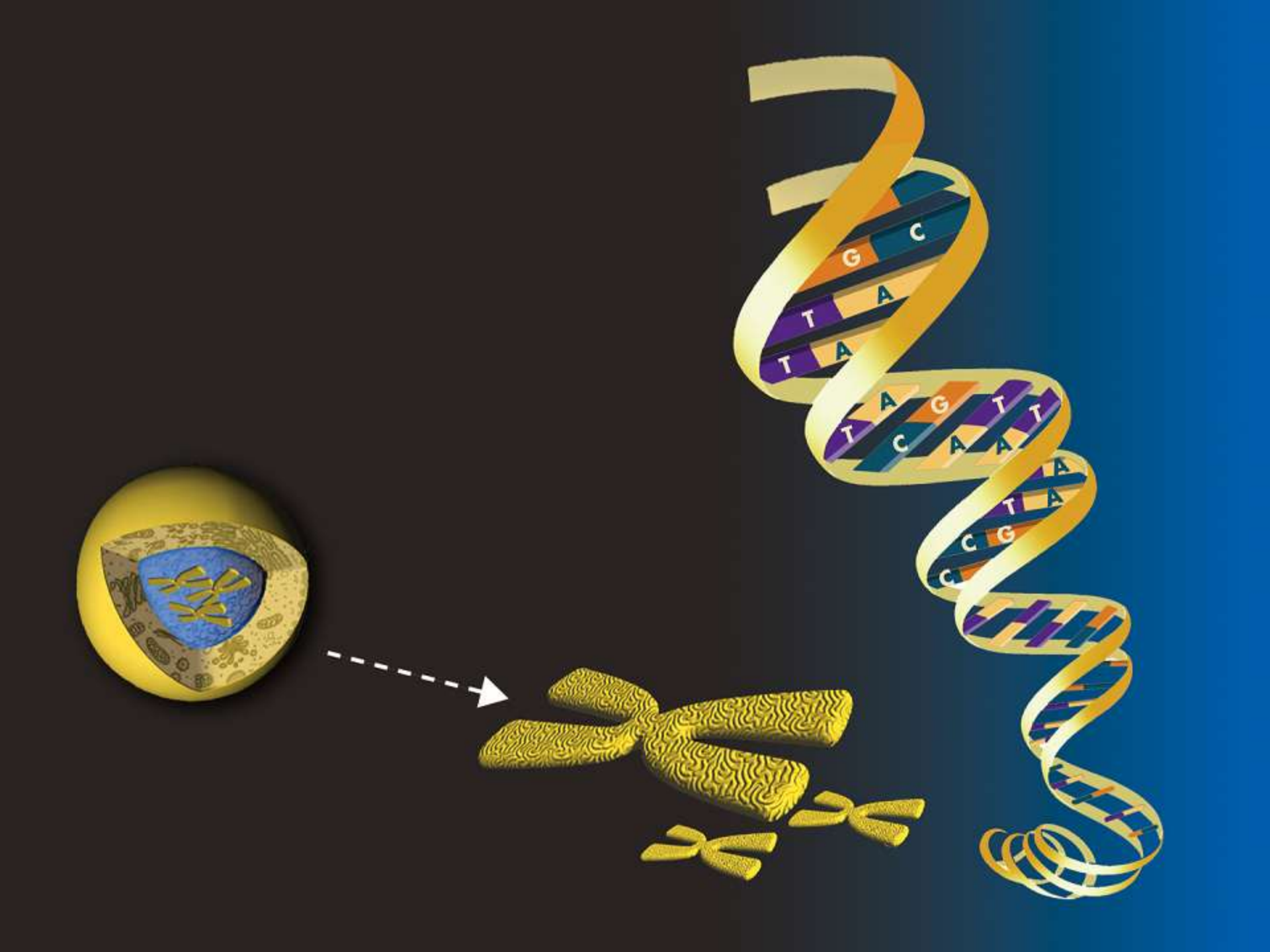
CA law to prohibit cloned pets ?

AB 1428, as introduced, Levine.

Commercial pet cloning. Existing law provides for the regulation of various types of businesses by the Department of Consumer Affairs. This bill would declare the intent of the Legislature to prohibit the commercial sale and transfer of cloned or genetically modified pet animals within California.

Manx cat ? Munchkin cat ?

GENETIC ENGINEERNG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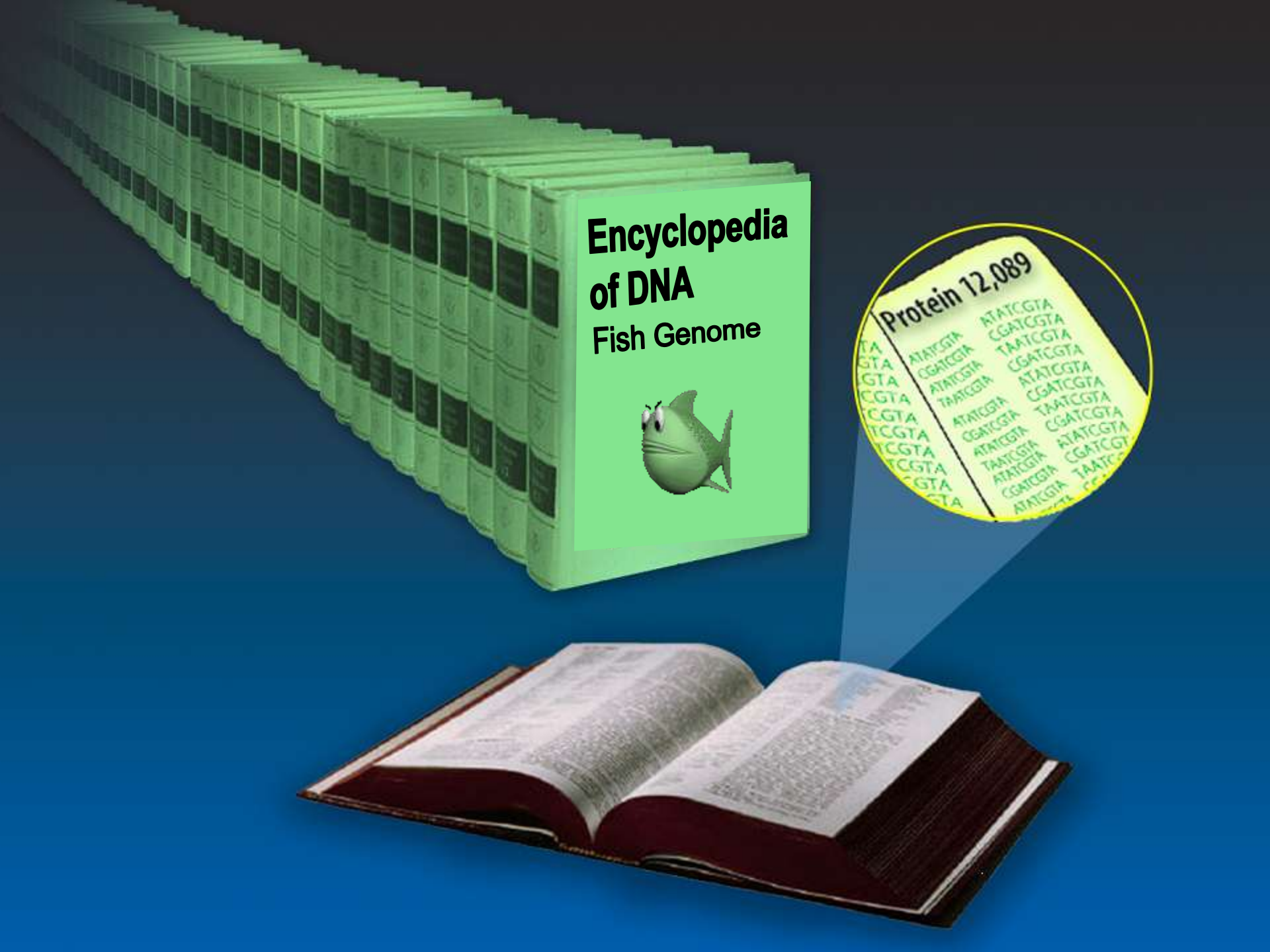
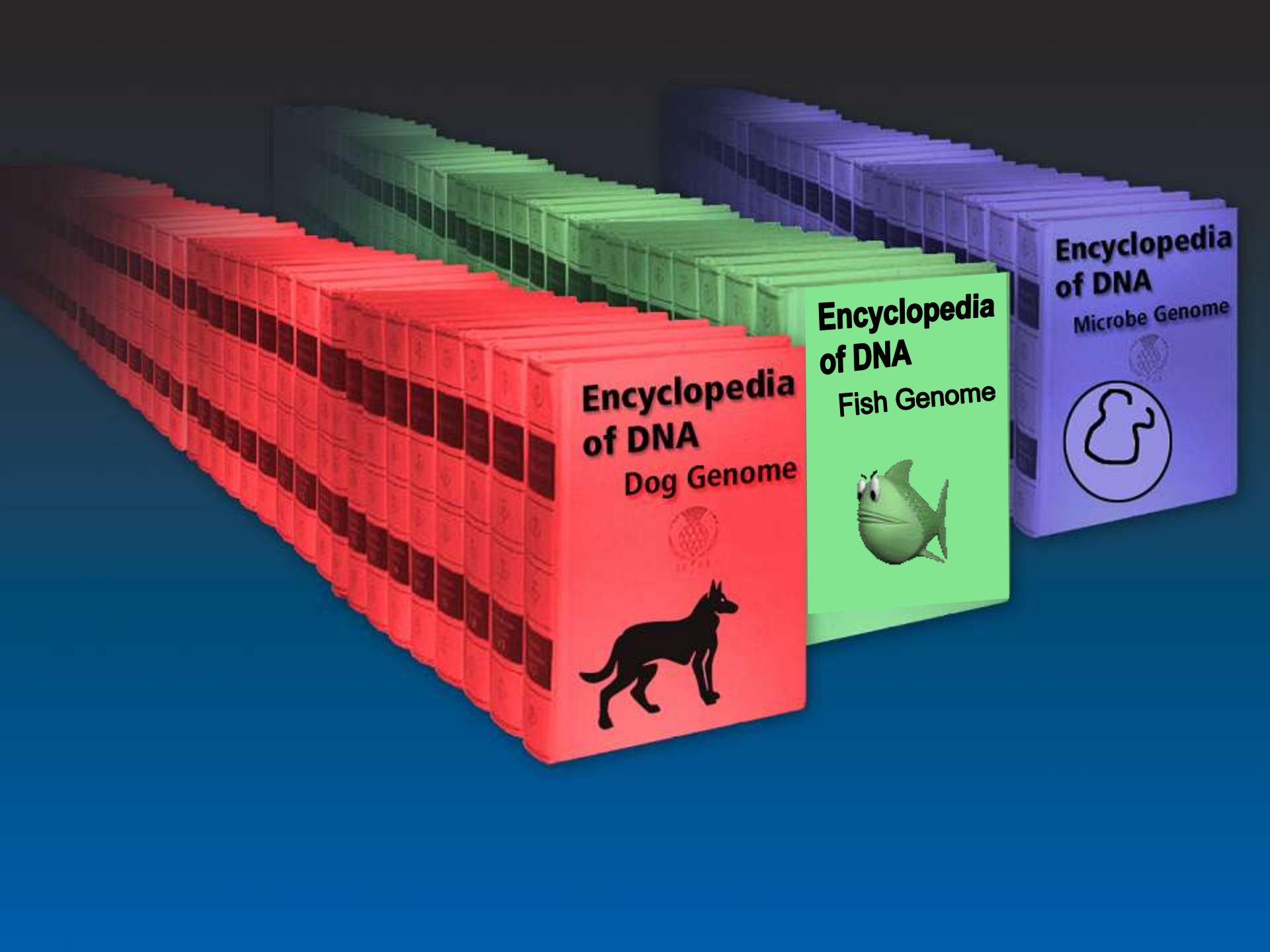
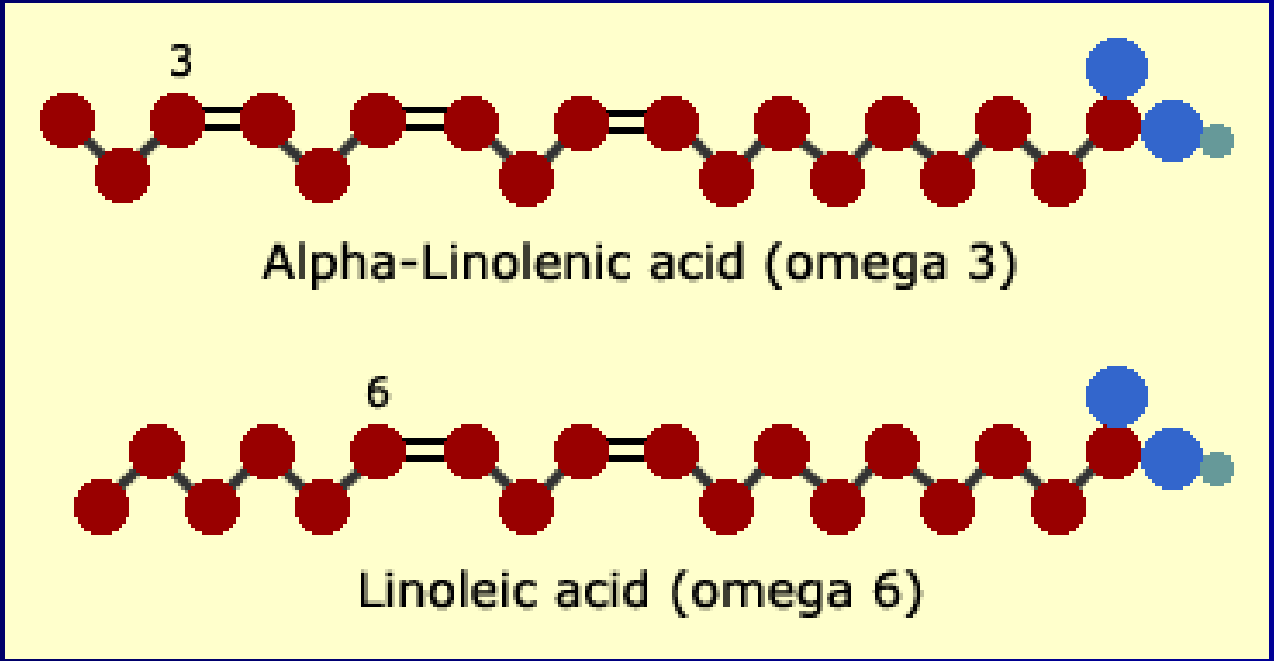
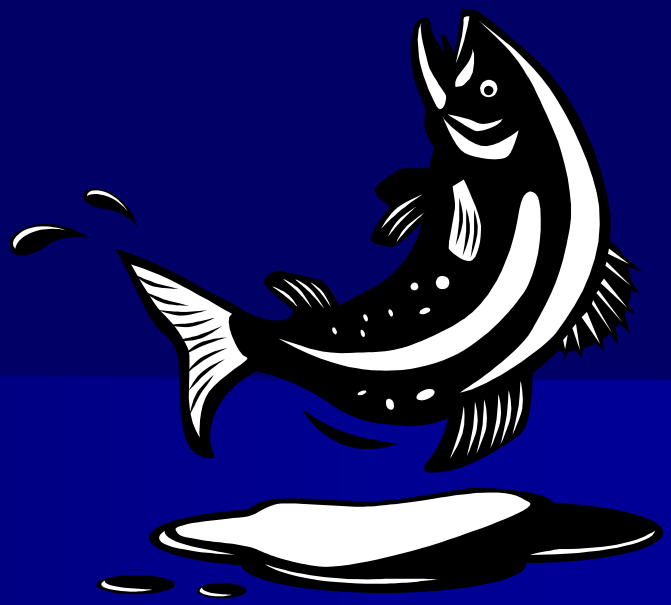

Encyclopedia
of DNA
Fish Genome
Protein 12,089


Encyclopedia of DNA
Dog Genome
Encyclopedia of DNA
Fish Genome
Encyclopedia of DNA
Microbe Genome

What is an omega-3 fatty acid ?



In the omega system the numbering refers to the first carbon with a double bond relative to the carbon at the methyl end of the molecule.



- The diet of our ancestors was higher in fiber, rich in fruits, vegetables, grazing livestock, and fish.
- The diet was less dense in calories, lower in total fat and saturated fat, and contained approximately equal amounts of *n*-6 and *n*-3 PUFAs
- In Western diets, the current ratio of *n*-6 to *n*-3 PUFAs is about 10 to 20:1, indicating that Western diets are deficient in *n*-3 fatty acids compared with the diet on which humans evolved

In USA, consumption and use of corn oil has progressively replaced and supplanted animal fats, so that corn and other vegetable oils now represent more than 70% of the PUFA dietary source

Profound quantitative and qualitative alterations in total FA intake over the past 40 years (France).

	Current intake g/day/adult (in 2000)	"ANC"[a] recommendations	40 years evolution (fold change)	Plant lipid-related increase (g/day)	Animal lipid-related increase (g/day)
Total FA	104	81	1.4	+18.8	+10.1
Palmitic acid (C16:0)	22		1.4	+1.3	+5.0
Oleic acid (C18:1)	33		1.2	+2.3	+2.2
Linoleic acid (C18:2 $n-6$)	21	10	2.5	LA +11.9	+1.0
Arachidonic acid (C20:4 $n-6$)	0.5		2.3		+0.3 ARA
Total $n-6$ PUFAs	22		2.5	+11.9	+1.3
α-Linolenic acid (C18:3 $n-3$)	0.9	2	0.6	+0.3	−0.9 ALA
$n-6/n-3$ PUFAs	12		2.9		
LA/LNA	23	5	4.2		

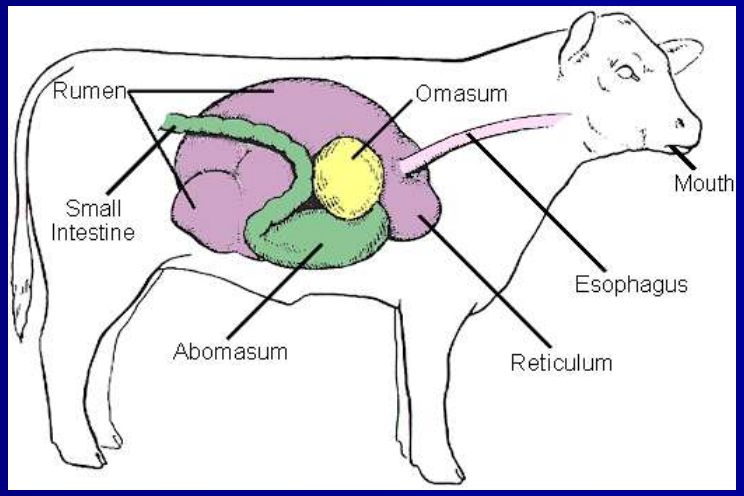
[a] ANC, "Apports Nutritionnels Conseillés", i.e. recommendations for intake.

The amount of ingested lipids has gone up 1.4-fold in the last 40 years, that of *n*−6 PUFAs 2.5-fold, and that of ARA (20:4n-6) 2.3-fold

Ailhaud, G., F. Massiera, P. Weill, P. Legrand, J. M. Alessandri, and P. Guesnet. 2006. Temporal changes in dietary fats: Role of n-6 polyunsaturated fatty acids in excessive adipose tissue development and relationship to obesity. Progress in Lipid Research 45:203-236.

Changes in the feeding pattern of livestock

For the same quantity of ingested fatty acids by the cow, a corn-based diet will contribute daily to 250 g of LA (18:2n-6) and 5 g of LNA (18:3n-3) cf. 60 g of LA (18:2n-6) and 250 g of LNA (18:3n-3) for a grass-based diet



Dramatic qualitative changes with respect to *n*–6 PUFAs in animal derived lipids

Despite the fact that complex biohydrogenation mechanisms of fatty acids take place in the rumen, changes in animal diets, LNA (18:3n-3) and *n*–3 very long-chain PUFA intake have much decreased. whereas that of ARA (20:4n-6) has increased 2-fold and the ***n*–6/*n*–3 PUFAs ratio has increased more than 4X.**

Table 11
Overall changes in France of the fatty acid composition of consumed lipids from terrestrial animal species (milks, eggs, meats) (expressed as % of total fatty acids)[a]

Year	Palmitic acid (C16:0)	Oleic acid (C18:1)	Linoleic acid (C18:2*n*–6)	α-Linolenic acid (C18:3*n*–3)	Arachidonic acid (C20:4*n*–6)	Long-chain *n*–3 PUFAs	*n*–6/*n*–3 PUFAs
1960	25	33	5.5	2.4	0.4	0.5	2.0
2000	29	31	6.2	0.6	0.7	0.2	9.1

Fatty acids as adipogenic hormones

- Polyunsaturated fatty acids (PUFAs) of the *n*–6 series, especially ARA (20:4n-6) are very adipogenic, meaning they promote differentiation of adipocytes (adipogenesis)
- One key question to be addressed in humans is whether the balance of PUFAs has changed during pregnancy and/or the lactation period such that it favors excessive adipogenesis during the early stages of adipose tissue development, i.e. during fetal life and infancy.

Infant "Obesity"

The percentage of US children between 6 and 11 *months* of age above the 95th percentile of the weight-for-length growth reference curve has increased in boys from 4.0% between 1976 and 1980 to 7.5% between 1988 and 1994, and in girls from 6.2% to 10.8% during the same periods of time.

The LA/LNA ratio of US breast milk has increased from the value of **6.0–8.0** before 1970 to **14–16** since 1980.

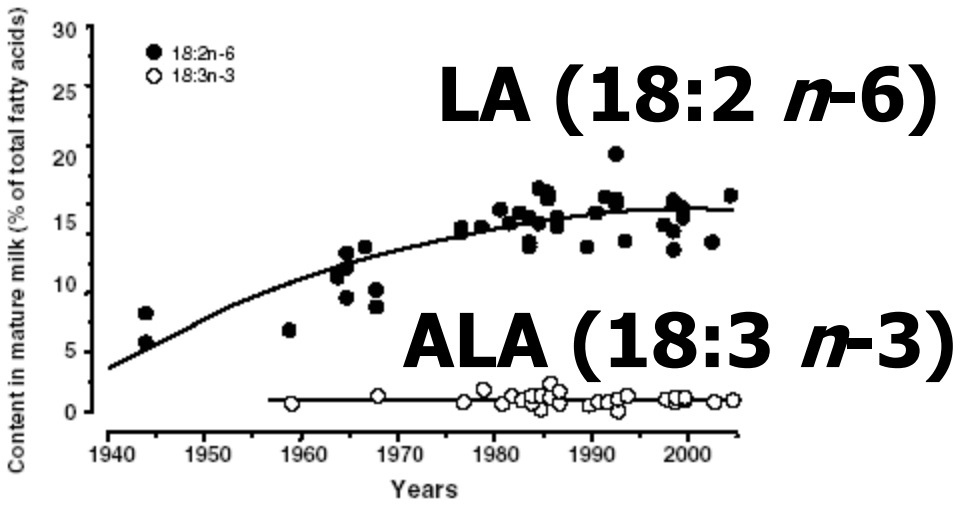
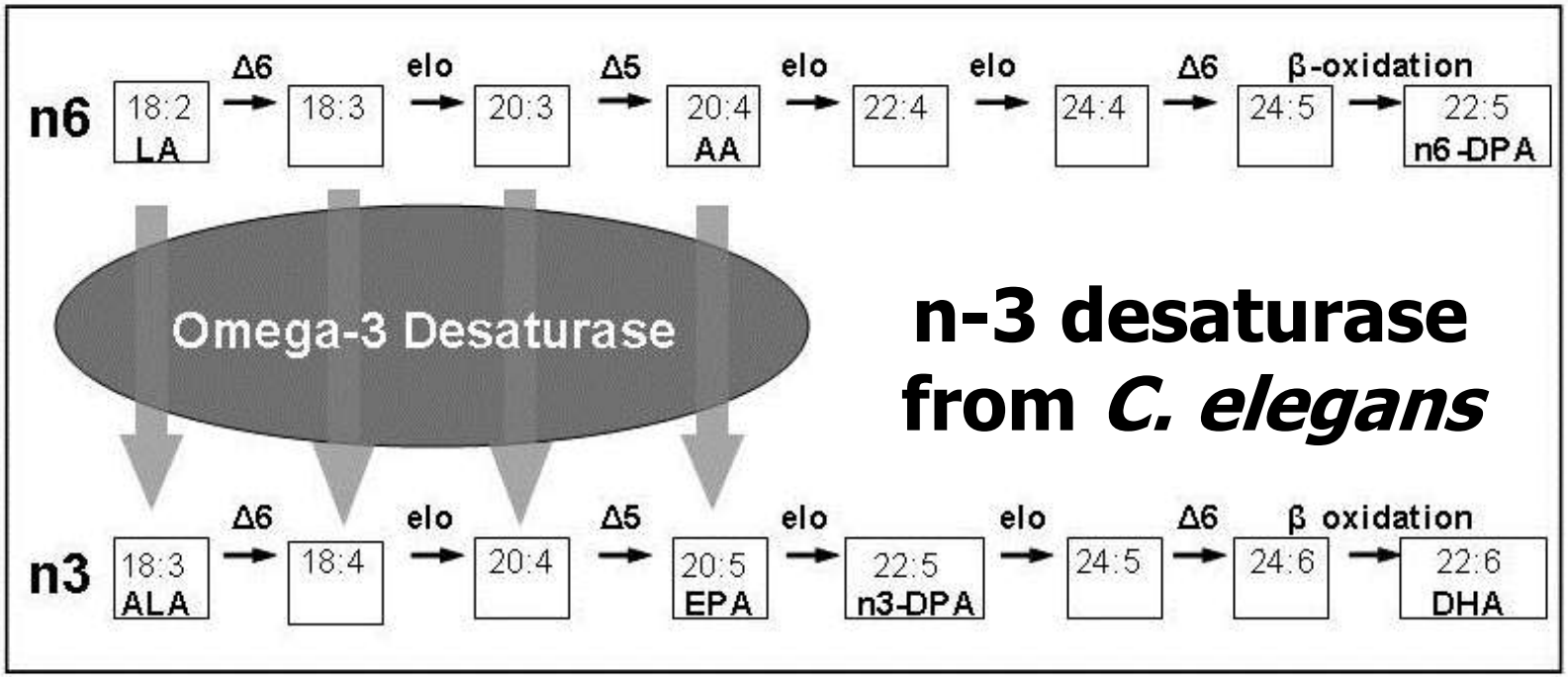


Fig. 3. Linoleic acid (LA) and α-linolenic acid (LNA) content in mature breast milk of US women from 1944 to 2005

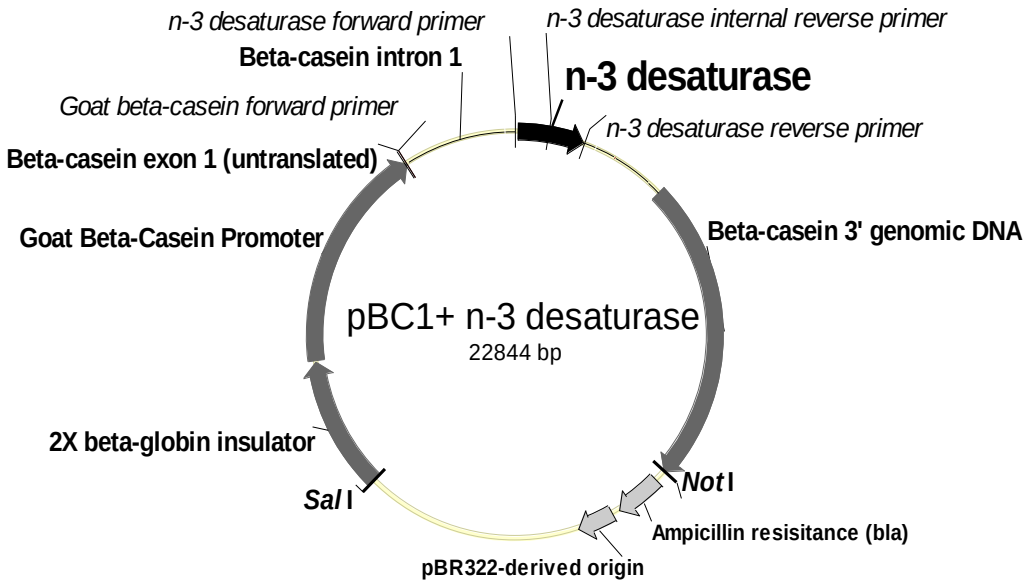
Develop a transgenic mouse model expressing omega-3 desaturase



B. T. Kao, K. A. Lewis, E. J. DePeters, and A. L. Van Eenennaam. 2006. Endogenous Production and Elevated Levels of Long-Chain n-3 Fatty Acids in the Milk of Transgenic Mice. Journal of Dairy Science. 89:3195-3201.

Development of the omega-3 milk mouse model

Beth Kao (MS)

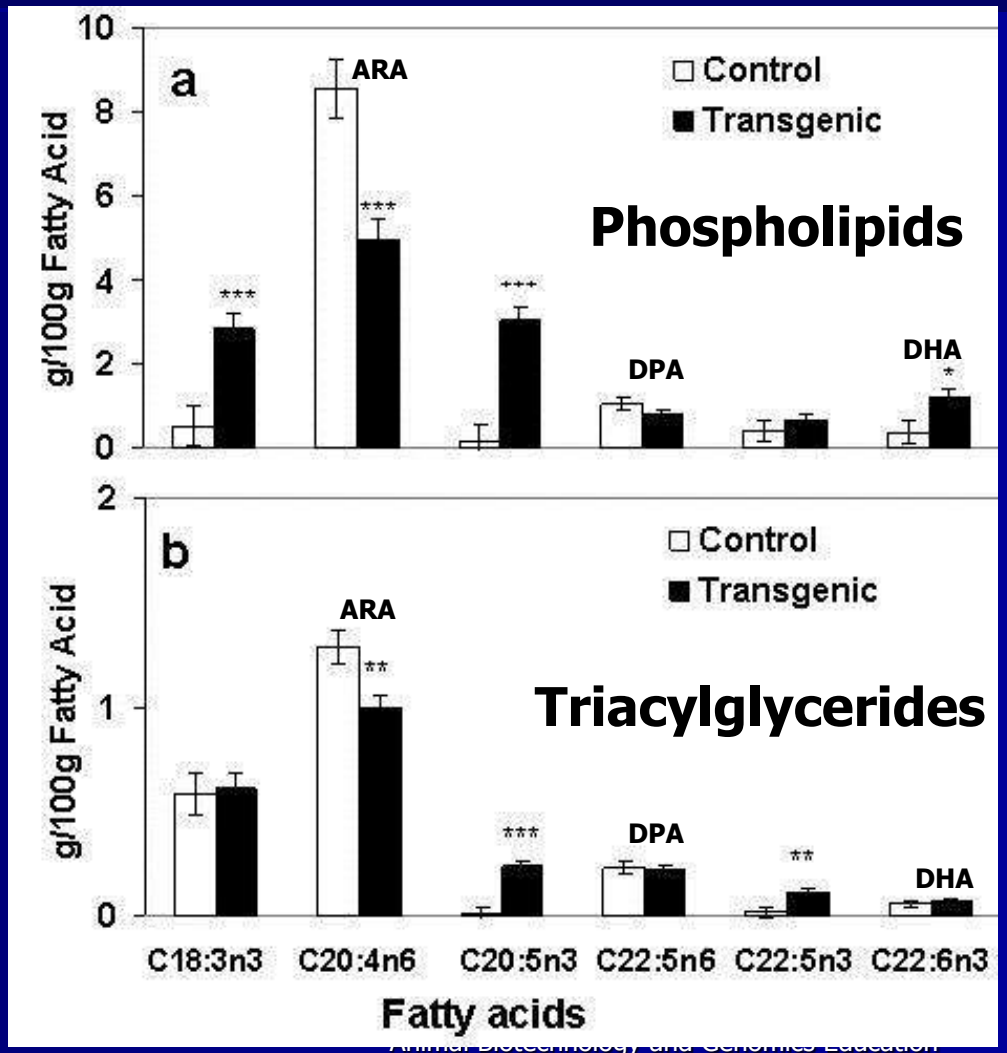


Transgenic mice were generated by pronuclear microinjection of the *SalI/NotI* fragment containing the *C. elegans* omega-3 fatty acid desaturase (Fat-1) under the control of the goat beta-casein promoter of the pBC1 mammary expression vector.

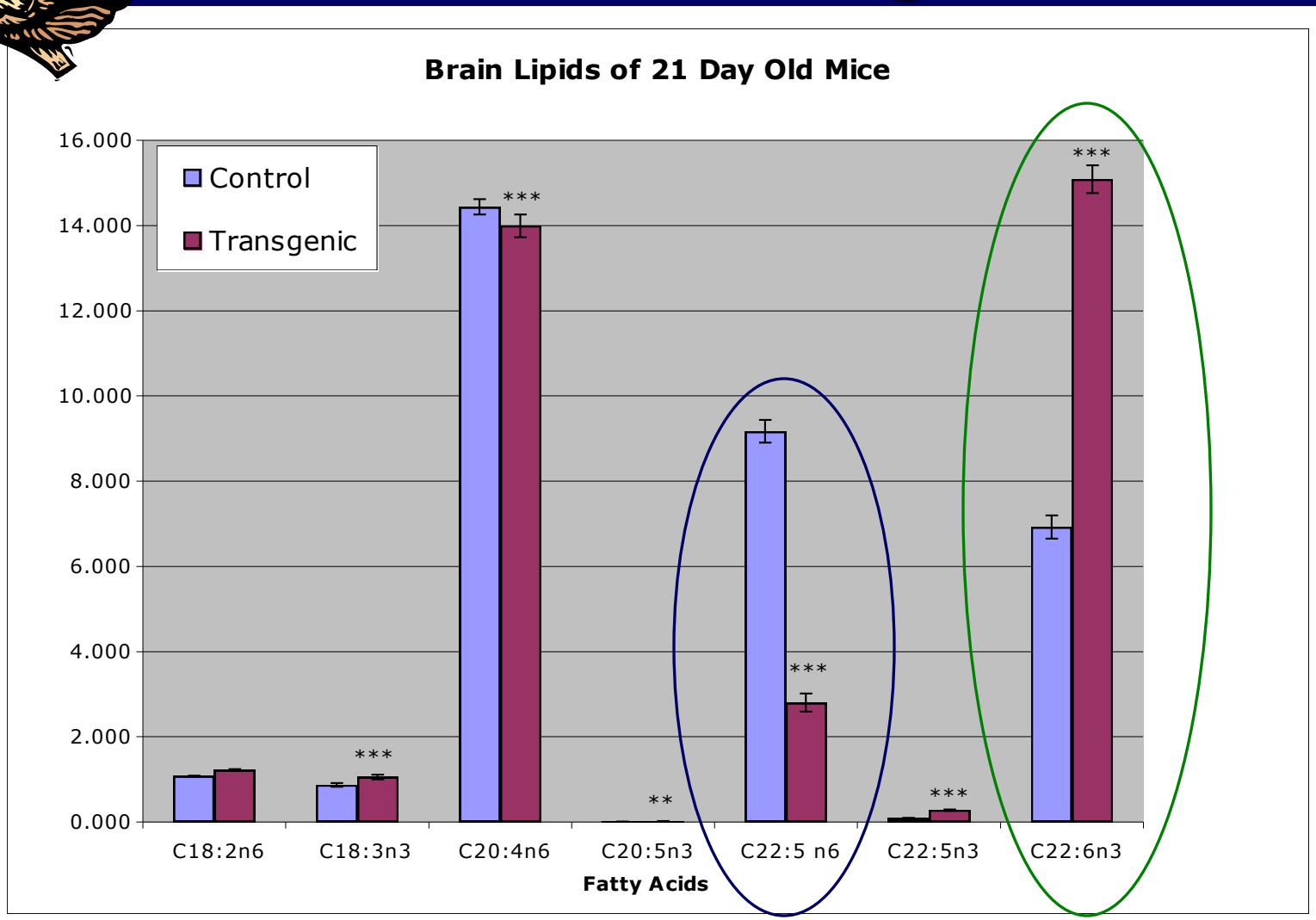
Milking day at the mouse house

What effect does nursing omega-3 dams have on pup phenotypes ?

- Transgenic females and nontransgenic full sib control females were placed on a high safflower oil (n-6) diet from breeding to 21 days post-parturition.
- There were 33 pups reared in each treatment group.



Brain lipids of 21 day pups nursed on control or transgenic dams

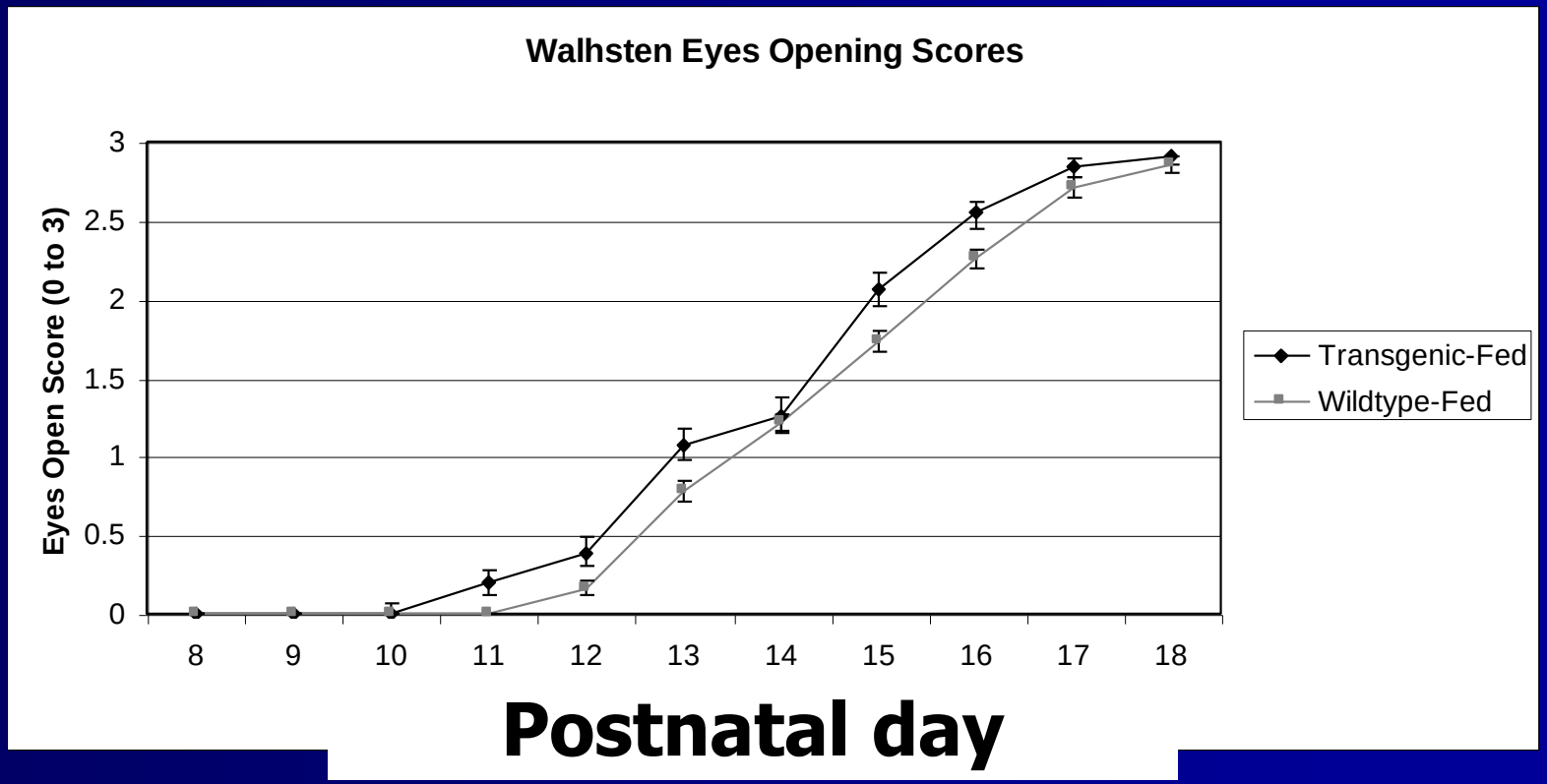


B.T. Kao, E. J. DePeters, and A. L. Van Eenennaam. 2006. Mice Raised on Milk Transgenically-Enriched with n-3 PUFA have Increased Brain Docosahexaenoic Acid. Lipids. 41(6):543-9.

Analysis of pup phenotype

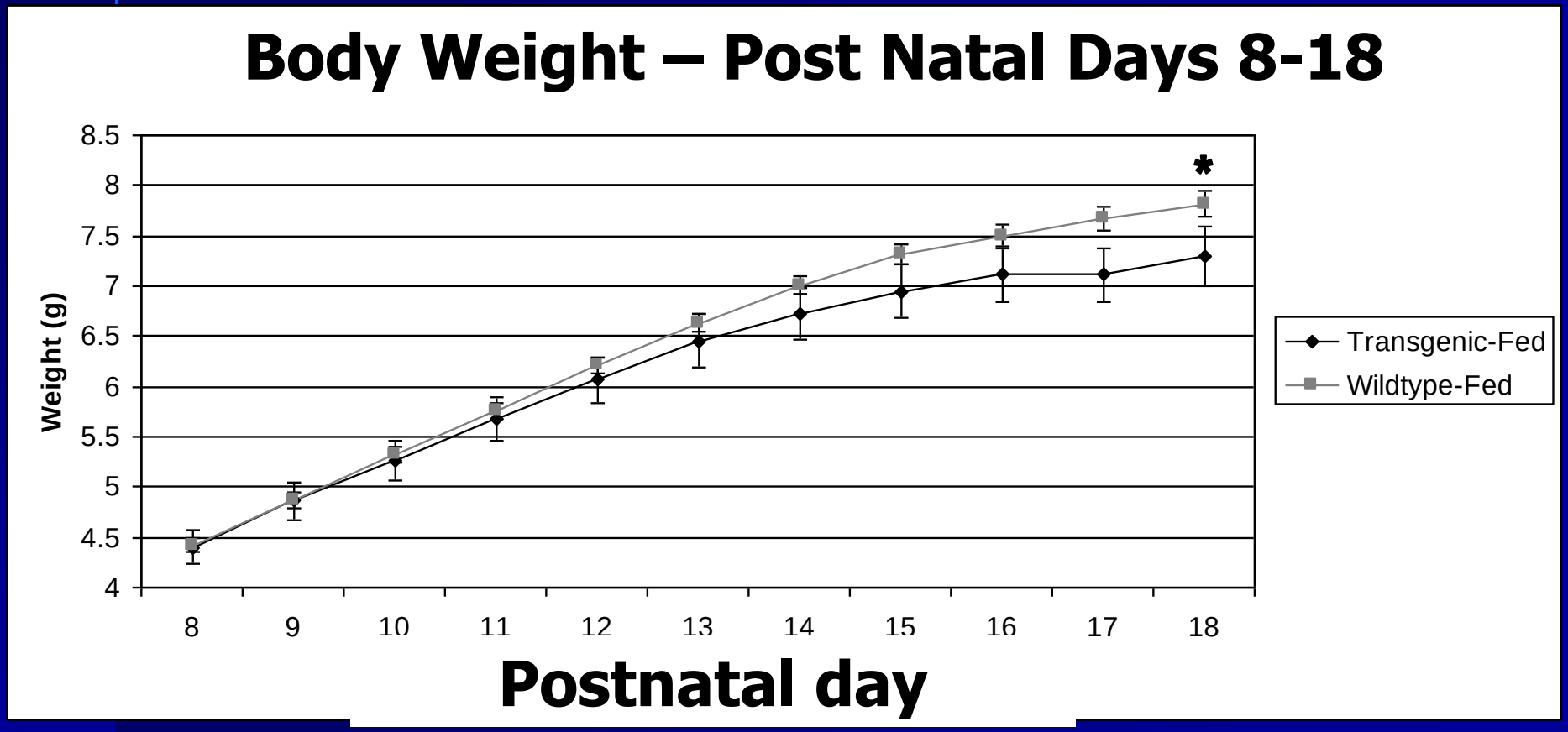
Kathleen Bongiovanni (MS)

DHA is known to favor the maturation of visual acuity

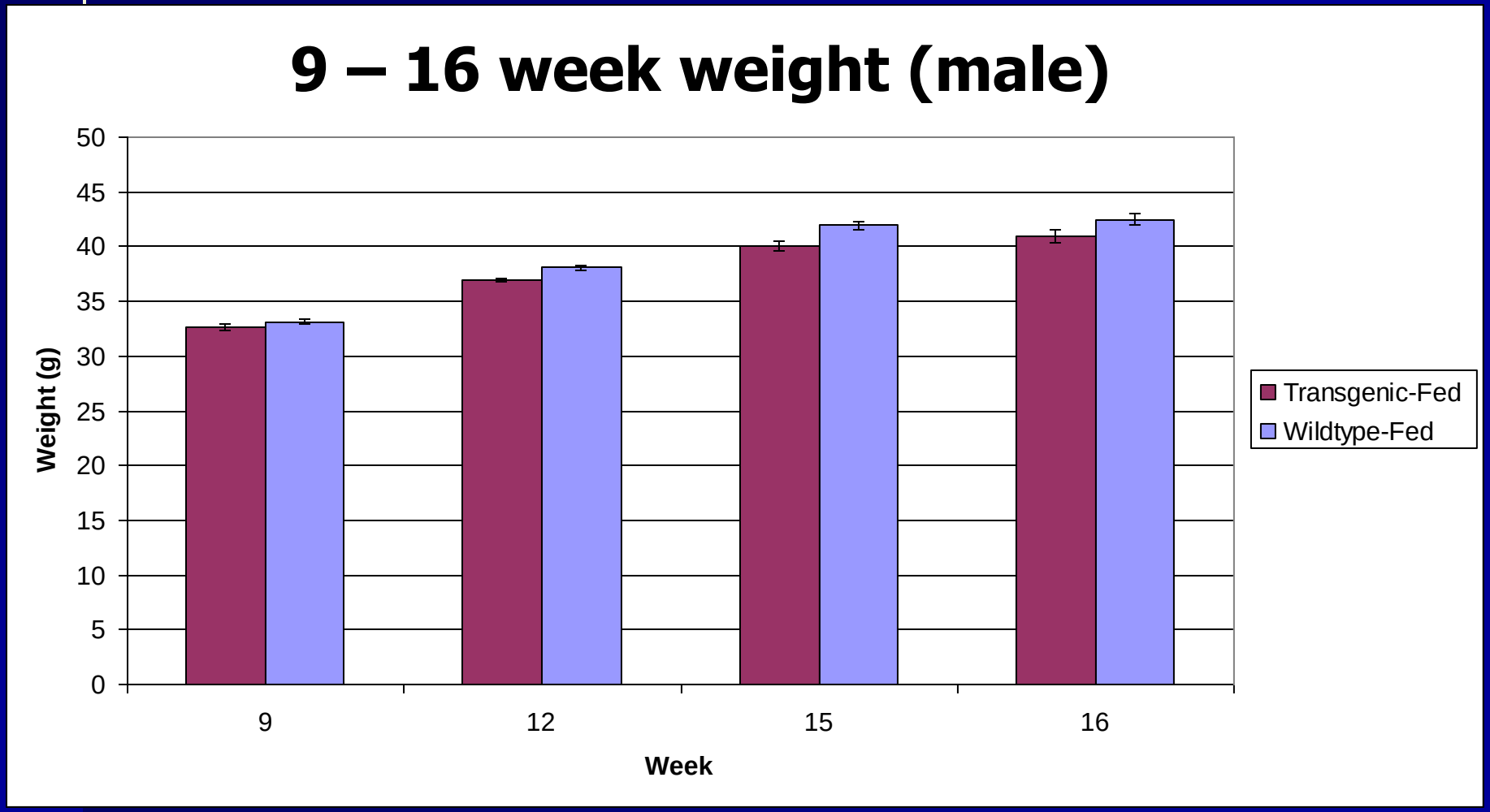


Bongiovanni, K. D. 2006. An analysis of the adiposity phenotype and cognitive benefits arising from the dietary supplementation of omega-3 fats via a transgenic mouse model. Unpublished M.S. thesis, UC Davis.

By the end of weaning the pups raised on transgenic dams were heavier than pups raised on omega-3 milk.



Weight of adult mice (n=21 or 26)



Breakdown of fat composition of carcasses

16 Weeks	Sex	N	Weight (g)	GFP (g)	FFP (g)	MFP (g)	RFP (g)	TF (g)	AI (%)	BMI
						*				
Transgenic-fed	M	21	40.95 (0.97)	1.83 (0.12)	0.55 (0.04)	0.73 (0.07)	1.05 (0.09)	4.18 (0.29)	10.20 (0.50)	44.08 (0.74)
Wildtype-fed	M	26	42.47 (0.89)	1.89 (0.11)	0.53 (0.03)	0.94 (0.09)	1.01 (0.07)	4.36 (0.28)	10.31 (0.55)	44.94 (0.65)
						*				
Transgenic-fed	F	20	35.35 (1.13)	2.05 (0.20)	0.50 (0.04)	0.78 (0.07)	1.25 (0.10)	4.58 (0.38)	12.72 (0.75)	40.19 (0.83)
Wildtype-fed	F	28	36.3 (1.03)	2.08 (0.16)	0.46 (0.03)	0.92 (0.08)	1.34 (0.09)	4.80 (0.34)	12.99 (0.64)	41.39 (0.80)

Data are mean (SEM).

GFP = gonadal fat pad weight

FFP = femoral fat pad weight

MFP = mesenteric fat pad

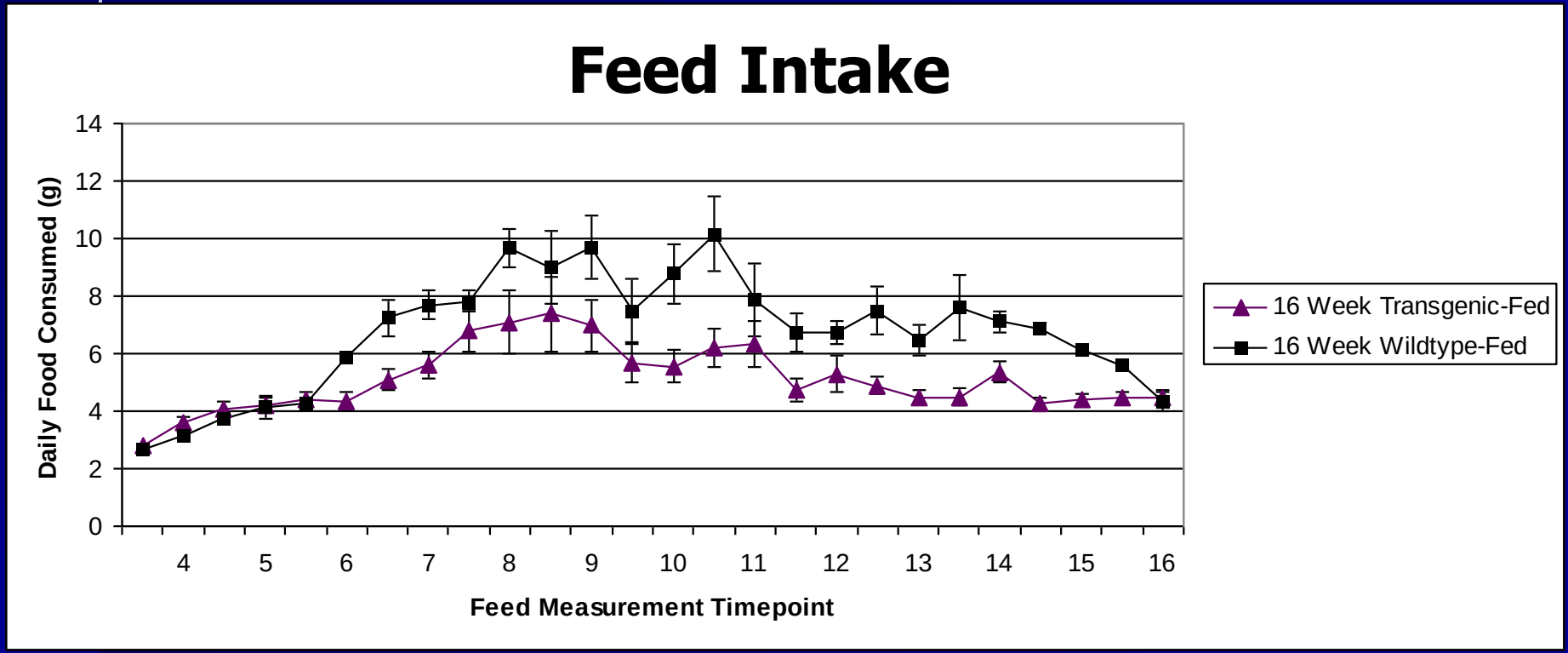
RFP = retroperitoneal fat pad weight

TF = total fat, summed weight of four fat pads

AI = adiposity index (TF/body weight*100)

BMI = body mass index (body weight at sacrifice/NA^2*100)

Post-weaning feed intake



Conclusion

- The balance between n-6 and n-3 PUFA during perinatal development affects adipogenesis and has lifelong metabolic consequences
- There may be some connection between the qualitative fatty acid content of ingested lipids during pregnancy and lactation, and infant formula and childhood obesity.
- Seed oils are a major reason for increased dietary LA (18:2n-6)
- Increasing the proportion of n-3 fatty acids found in ruminant products offers a way to improve the nutritional content of an important component of the American diet, and would provide a compelling example of how industry is interested in the production of functional foods for the enhancement of human health.

Genetically engineered food animals

Enviropig™ (Low-phosphorus manure) http://www.uoguelph.ca/enviropig/

Nature Biotechnology, 19, 741 - 745, (2001).

Transgenic cows expressing an antibacterial endopeptidase in their mammary glands show enhanced resistance to mastitis.

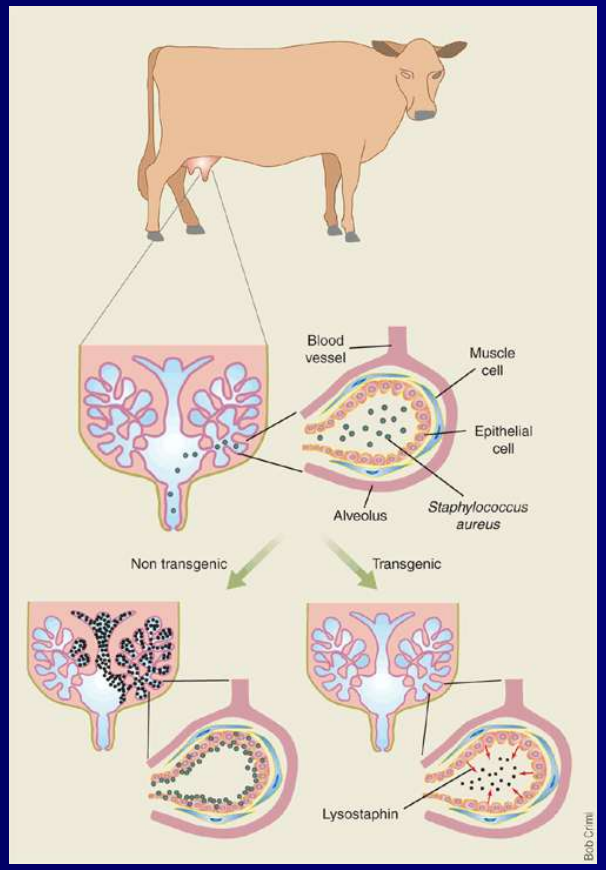
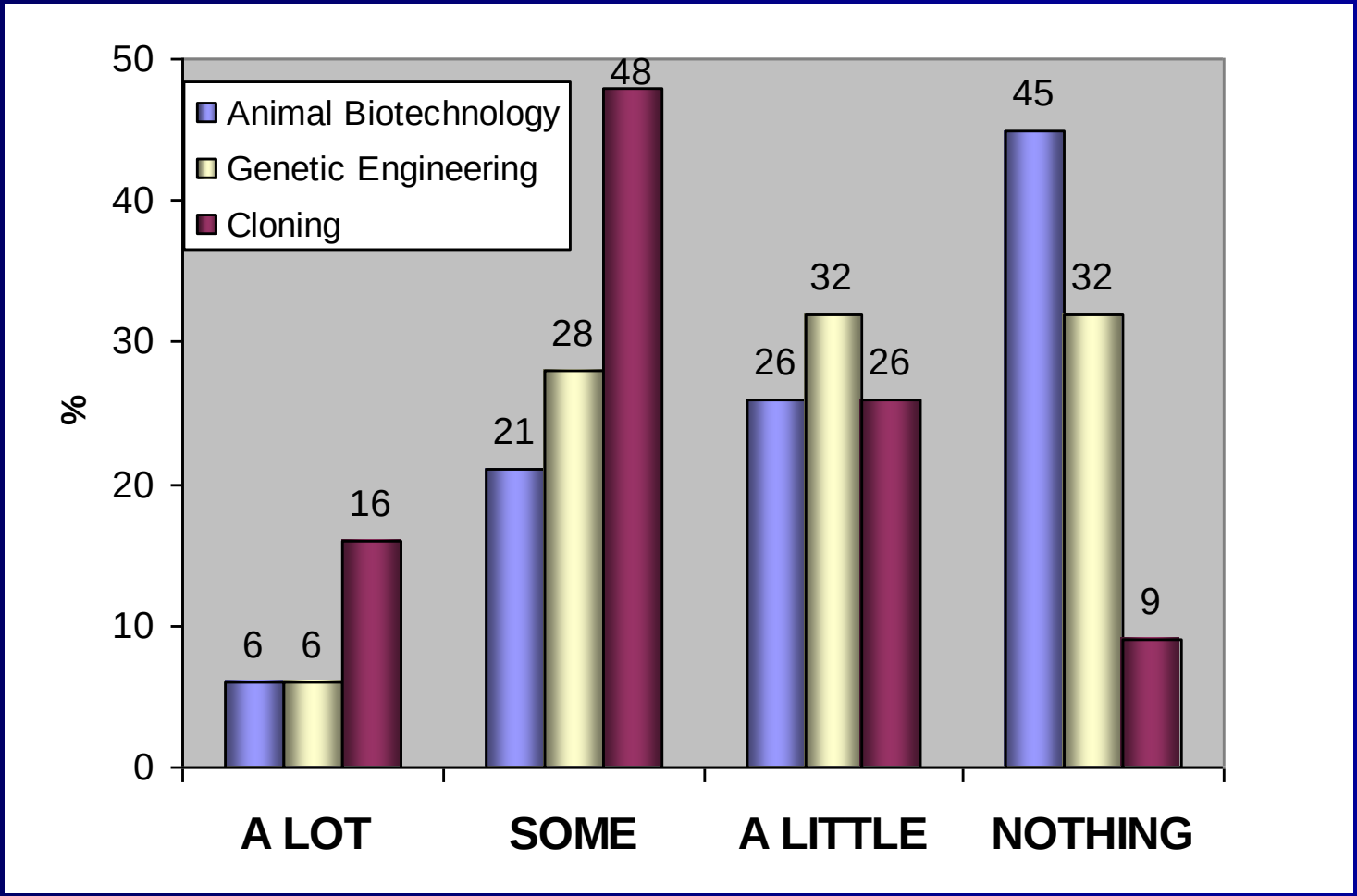


Figure 1. Staphylococci enter the mammary gland through the teat canal and use the milk as a nutrient to multiply and as a vehicle to disseminate in the ducts and alveoli of the secretory tissue. *In a normal mammary gland (lower left), the bacteria injure and colonize the epithelium, which triggers the inflammatory response. In mammary glands whose epithelial cells secrete lysostaphin (lower right), staphylococci are killed before they can damage the mammary tissue and induce inflammation.*

Wall,R.J. *et al.* Genetically enhanced cows resist intramammary Staphylococcus aureus infection. *Nature Biotechnology* **23**, 445-451 (2005).

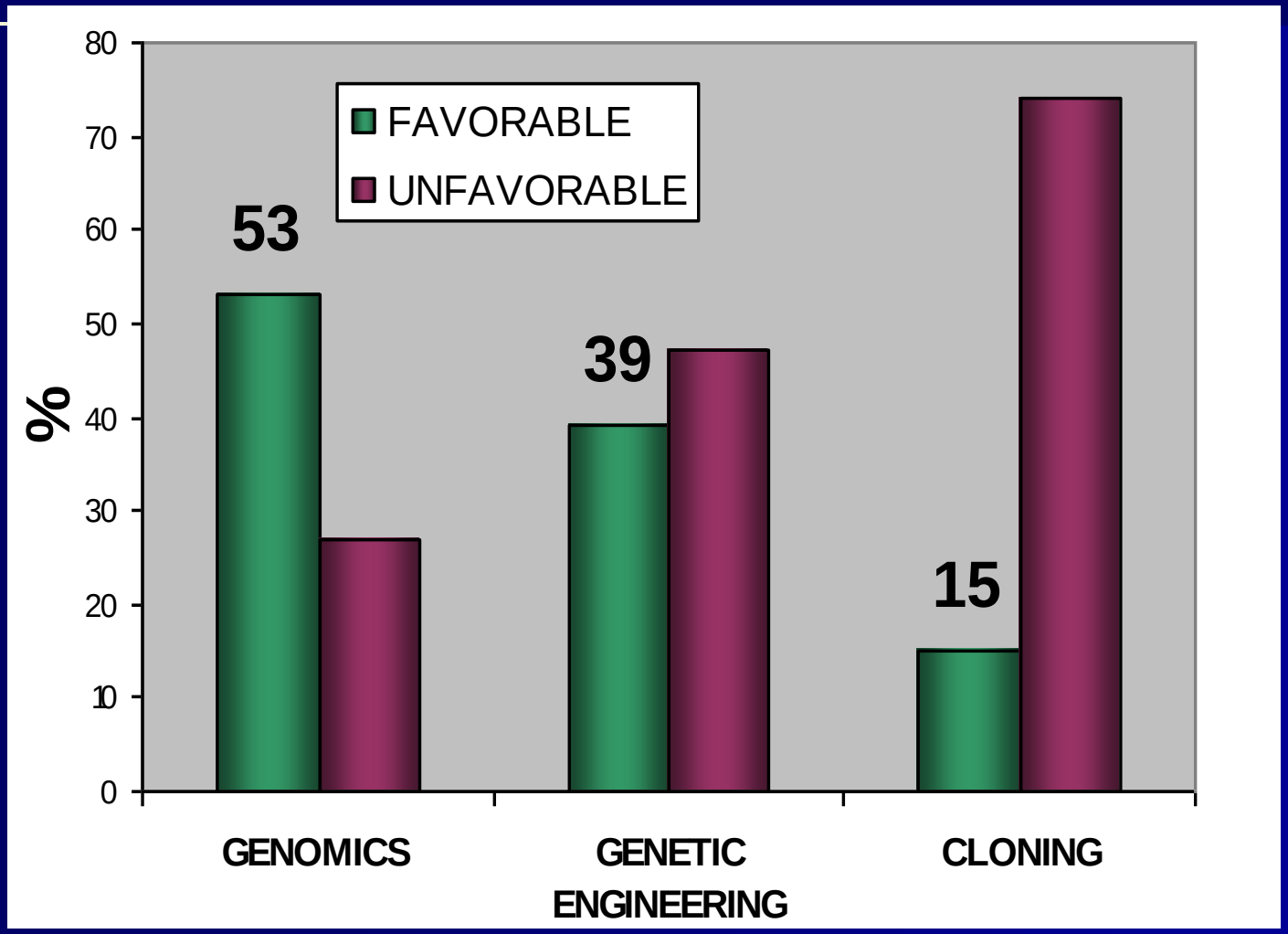
US Public Attitude Surveys

How much have you heard about specific animal biotechnologies ? (IFIC + PEW, 2005)



http://ific.org/research/upload/2005BiotechSurvey.pdf

Public Attitudes Towards Specific "Animal Biotechnologies" (IFIC, 2005)



http://ific.org/research/upload/2005BiotechSurvey.pdf

Commercialized products of animal biotechnology….

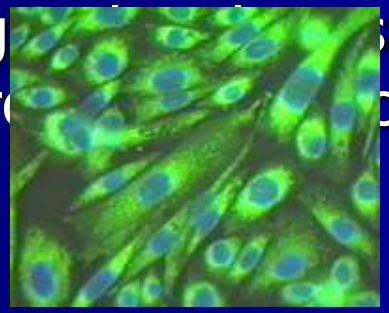
GloFish™

Genetic Savings&Clone, Inc.
GENE BANKING & CLONING OF EXCEPTIONAL PETS

Summary – and lessons for genetic engineering of animals

- Genetic engineering of animals is going to be a hard sell
- Applications for health and medical benefits viewed most positively and although support is strongest for pharmaceutical production uses (49%), the public is more supportive of the use of GE plants (81%) for this purpose .
  - $100,000 annual price for Avastin (a humanized monoclonal antibody produced in an engineered CHO cell line) to treat breast or lung cancer - Genentech
- enerally opposed to ag – e ortive of disease-pr cat eding pets.

CHO Cells



http://pewagbiotech.org/research/2005update/2005summary.pdf

GloFish™ – barred on ethical grounds in California - "just a pet"

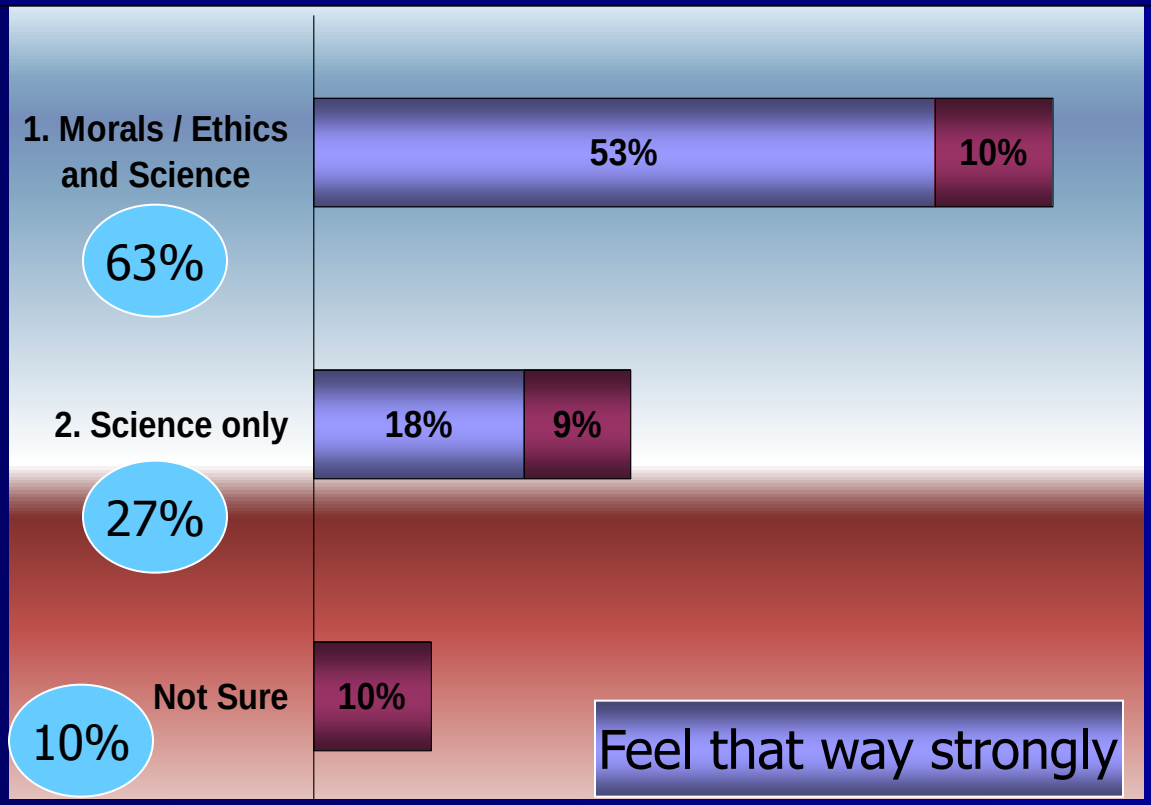
“just a floral arrangement”

GE animals raise unique moral, ethical, and cultural questions

- Animal "integrity"
- Animal welfare
  - related to breeding goals
  - related to biotechnology
- Environmental issues with regard to gene flow from GE animals to native populations.

1. Government regulators ***should include ethical and moral considerations***, in addition to scientific evaluation of risks and benefits, when making regulatory decisions about cloning or genetically modifying animals.

2. Though ethical and moral considerations are important, government regulators ***should consider only scientific evaluation of risks and benefits*** when making regulatory decisions about cloning and genetically modifying animals.



http://pewagbiotech.org/research/2005update/2005summary.pdf

How to incorporate social and ethical issues into regulatory decisions ?

- American consumers (75%) and scientists (70%) agree that cloning and genetic engineering of animals raise some moral and ethical issues
- However public is much less likely to approve (21-25%) of these technologies than scientists (60-68%)
- How to reach a societal consensus on ***which set of values*** will ultimately be applied to decide the acceptable uses of animal biotechnology ?
- Incorporation of "non-technological concerns" is the most difficult issue faced by animal biotechnology

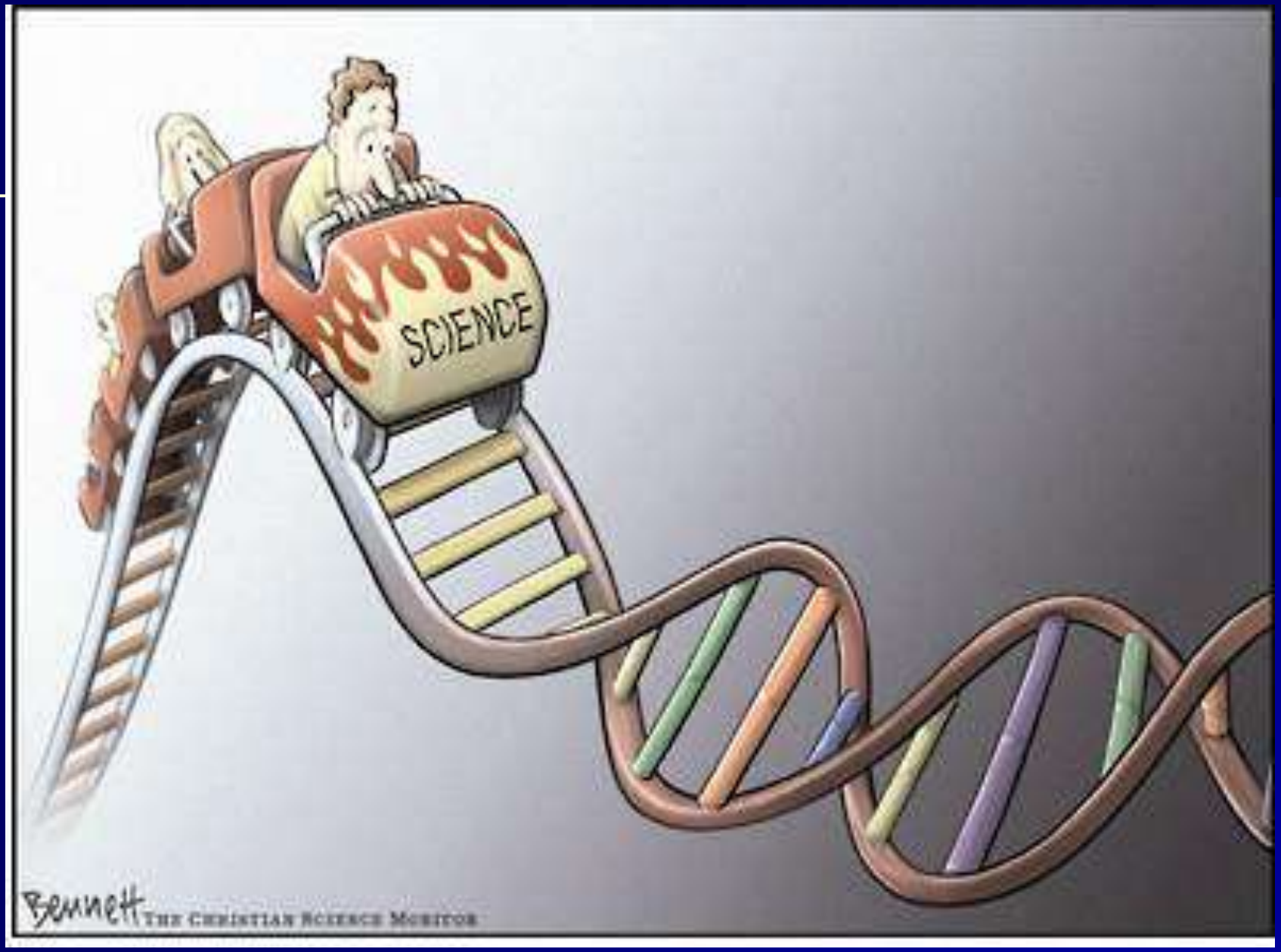
Keystone Research Center (2004) – Biotechnology and ethics: a national survey of consumers and scientists. Report to the Biotechnology Industry Organization. KRC Research, Washington DC, 29pp.

Dualism (right versus wrong) is the enemy of compromise

As long as groups polarize the issue of genetic engineering into “all is permitted” or “nothing is permitted”, rational social progress on the issue is impossible. Dualism, or dilemma thinking (right versus wrong), is the enemy of compromise and the archenemy of the middle way.

SUMMARY

- No GE or SCNT cloned food animals currently on the food market
- FDA regulates GE/cloned food animals
- Future uses of GM animals could be diverse and may address important societal needs
- Yet to see if the expense of the technology is commercially viable
- GE animals raise unique moral, ethical, and cultural questions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