and be observed closely for signs of respiratory failure. Antimicrobics may be given for specific secondary complications (i.e., aspiration pneumonia). Antimicrobics have been ineffective in eradicating the organism from the intestinal flora (Beaty 1983). If antimicrobics are used, those that may potentiate neuromuscular weakness should be avoided, i.e., aminoglycosides, tetracyclines, and procaine penicillin. After the specific antitoxin is administered, continued absorption from the intestine should have minimal or no additional effect on the clinical condition. Additionally, some form of cathartic usually is recommended because of the ileus. Magnesium products should be avoided, as these may potentiate the neuromuscular weakness. Mineral oil or sodium sulfate may be used as cathartics. Parasympathomimetics, although they may provide temporary improvement in clinical signs, have been shown to increase mortality. Adherence to general principles of good nursing care and nutrition are of utmost importance because of the prolonged time required for recovery. Recovered patients are thought to regain normal nervous and muscular function.

In each of these outbreaks Type B organisms were isolated from the silage which may have been improperly fermented. The key factor may be the lack of adequate fermentation to reach a low pH, thus inhibiting sporulation and toxin production by *Clostridium botulinum*. Botulinum organisms were also isolated from the rumen contents and feces of affected cattle which help confirm the diagnosis because normal cattle do not have botulinum organisms in the gastrointestinal contents. The exception to this is the animal at risk, i.e., exposed to a herd outbreak but not showing clinical signs. Botulism does occur sporadically and the practitioner needs to be kept aware of that possibility.

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Engineering Tomorrow's Cow: Embryo Sexing, Splitting and Gene Insertion

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Introduction

We live in a world of technology, including biotechnology such as genetic engineering. While several "buzz" words have become popular recently, we have lived in a world of biotechnology for at least a generation.

Artificial insemination (A.I.) is an example of the greatest single biotechnology applied to livestock, particularly dairy cattle (1). This opened the field of sperm physiology and the study of fertilization and embryo mortality, as well as the control of certain infectious diseases. Artificial insemination and dairy record keeping provided information for the geneticists to develop effective dairy sire selection programs. Computers were used extensively, starting in the 1950's.

The development of frozen semen, with glycerol as a cryoprotectant, heralded the beginning of a major emphasis on cryobiology—freezing blood, tissues and organs followed. Today, this has developed so that frozen embryos

can be transferred successfully (6), making possible the long-time preservation of endangered germ plasm.

Embryo transfer itself was only an experimental tool used by researchers until the early 1970's (3). Then there was interest in expanding exotic beef breeds using surgical transfer techniques. With the development of nonsurgical procedures and the availability of prostaglandin F_2 alpha and various analogs, embryo transfer grew in dairy cattle. Last year about 100,000 fresh embryos were transferred and more than 20,000 frozen embryos were estimated to be transferred into North American cattle.

Embryo Sexing

Obviously it would be desirable to sex spermatozoa and produce all embryos of the desired sex. This would be a cheaper way of obtaining a sexed embryo; it could be done in major semen processing centers and would not result in production of embryos of the unwanted sex about 50% of the time. Dairymen usually would desire to obtain female offspring. A.I. organizations would want males to obtain sons for testing and daughters to test sons for milk production. As far as I am aware *there is no effective means of sexing bull sperm (2).* There are reports of producing more males in humans by a sperm sexing procedure.

Sexing embryos. Work by White et al. (10, 11) has demonstrated that embryos can be sexed with about 80 to 85% accuracy, using a male-specific antibody which was conjugated with the green fluorescent dye, fluorescein isothiocyanate (FITC). With this technique 85% of the bovine embryos which fluoresced had male (XY) karyotypes and 15% had female (XX) karyotypes. Only 3% of the non-fluorescing embryos were male (XY).

If one uses complement, then about half of the embryos are killed. In studies with mouse embryos 81% of non-affected embryos were found to be females upon transfer. In FITC studies, 78% of the FITC positive mouse embryos were males and 83% of the FITC negative embryos were females. Thus, it appears that with the right male specific antigen sexing can be about 80% accurate. FITC-treated embryos retain their viability following treatment so both positive and negative embryos can be transferred, if desired. For example, two male bovine embryos or two female embryos could be transferred to one recipient. If twins resulted freemartins would be avoided.

Bisection of Embryos

In some of the earlier work about half of the embryos bisected as blastocysts produced pregnancies. Williams et al. (12) bisected 72 embryos, and the 144 half-embryos produced 72 pregnancies. This was a 50% pregnancy rate but represented 100% in terms of the number of whole embryos available initially. Recently (Hasler, Em Tran, personal communication) indicated that as many as 150 pregnancies could be obtained from the split halves of 100 whole blastocysts. This is a 75% pregnancy rate. This reduces the cost of producing each pregnancy. Many sets of identical twins can be produced for commercial use and for research. Such twin embryos could be transferred to one recipient, even if the sex was unknown without producing freemartins.

One member of a bisected embryo can be frozen and the other one transferred. If an embryo has been accurately sexed as a male, an A.I. organization could test genetically one-half of each embryo following transfer to produce bulls. Those bulls that proved to be outstanding might be duplicated by thawing out the other half of each embryo. If a pregnancy resulted upon transfer a genetically identical twin should result.

Freezing of high quality embryos (6) has reached the point where pregnancy rates are high, if high quality embryos are frozen soon after collection (8). Generally, pregnancy rates have been reported for frozen embryos that are about 10% lower than results with fresh embryos.

Gene Insertion

The possibility of immediately modifying the gene complex of an animal by inserting genetic material into recipients has stimulated much excitement. Already insulin and growth hormone genes have been inserted into bacteria to produce large quantities of these two hormones. These hormones have widespread application. In cattle, Bauman et al. (1985) have reported almost immediate increases of 20 to 30% in milk production by the daily injection of around 30 mg. of the hormone. This is a major increase in the efficiency of production.

The injection of a "gene" into embryos so that it will be distributed to appropriate tissues, expressed and regulated in the recipient and transmitted to offspring is much more difficult (4, 7, 9). Even the microinjection of tiny volumes of genetic material into the pronuclei requires much skill. A major test has been reported by Hammer et al. (4) in a collaborative study with growth hormone (Table 1). The actual expression of this gene is low and not well controlled. The limited number of animals produced so far indicates both promise and the need for much more research before one can produce animals that are useful commercially. In the meantime valuable information can be learned from the study of these animals.

TABLE 1. Integration of a Metallothionein-I promoter-growth Hormone Construct Following Insertion into Embryos.

Species	Number of ova injected	Number of recipients	Integration frequency
Rabbits	1,907	73	12.8%
Sheep	1,032	192	1.3%
Pigs	2,035	64	10.4%

When genes become better located on chromosomes it will be possible to isolate these complexes in domestic animals. Many of the techniques used now with mice will be useful for large animal work in the future. An example is the work with

growth hormone (4, 9). Not only do genes need to be identified, but they must be transferred, integrated, expressed and regulated without deleterious effects. This will require much more research, especially for traits affected by multiple genes.

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Epidemiology of Bovine Salmonellosis

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Salmonellosis is a disease with great economic and public health impact. The burdens placed upon the animal industry each year due to salmonellosis amount to millions of dollars. Depending upon which salmonella serotype is involved in the disease process, there are also other associated problems: high morbidity in affected herds, abortion sequels, the problems of carrier cattle and the reoccurrence of salmonellosis in herds. Moreover, survivors of outbreaks are often unthrifty and take longer to reach marketable weight or do not regain lost milk production. Along with the lost revenues from decreased milk production are the costs of therapy and replacement animals, and public health problems leading to restrictions on the sale of milk and/or meat products.

Because of the ubiquity of the many salmonella serotypes in the environment, water, birds, rodents, etc., in feed stuffs that are easily contaminated, and because carrier animals are common, it is unlikely that salmonellosis will be eradicated. The best defense against salmonellosis, then, is prevention and control. Our efforts must be directed toward understanding the epidemiology of salmonellosis with the aim of breaking the cycles of infection. This research summary is a brief discussion of the epidemiology of salmonellosis in cattle, including the effects of stress and infection, the changing pattern of salmonellosis in cattle, and the use of modern epidemiological techniques that enable the fingerprinting of strains of Salmonella.

Epidemiology

The general epidemiological pattern of bovine salmonellosis is depicted in Figure 1. In many outbreaks it is not possible to definitely identify the source(s) of herd infection because site investigations are often conducted well after an outbreak has started (11, 12). Sometimes, however, through extensive bacterial cultures of animals, feed, water, and the environment early in an outbreak, a source may be identified (9). A cow may become infected in many ways. Once infected an acutely ill animal can rapidly contaminate the environment and serves as a focus to infect other cattle (24). The resulting widespread environmental contamination can make it very difficult for the epidemiologist to sort out the problem.

FIGURE 1. Epidemiology of Bovine Salmonellosis — Adult Cattle.