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Industry Application of Technology in Male Reproduction

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Introduction

Commercial artificial insemination (AI) is one of the greatest animal biotechnologies to have been introduced to the animal industry in the past century. The use of AI has resulted in significant genetic gains in the turkey, swine and dairy industries, while at the same time it has helped reduce disease transmission and accidents caused by sires. Currently 100% of turkeys and approximately 70% of the pigs (Estienne and Harper, 2005) and dairy cattle (DeJarnette and Marshall, 2007) in the U.S. are bred using A.I. In the beef industry, only about 10% of the cows are bred using AI (Seidel, 2003; DeJarnette and Marshall, 2007), and the question may be asked, why hasn't the beef industry taken advantage of the biotechnology more effectively?

Two important aspects of the genetic gains seen in the industries which utilize AI include the ability to utilize spermatozoa only from males possessing the best genetics and because males produce many more spermatozoa than is needed for a single insemination, more females can be bred with spermatozoa from these best males using AI than can be bred using natural service. Since single ejaculates can be split into many inseminations, fewer males are needed to breed a herd or flock of females; therefore, the selection intensity on the relatively few males that are kept can be increased even more. Using this strategy, milk production has increased from an average of 4,622 pounds/year in 1940 to 20,267 pounds/year in 2007 (USDA NASS milk production).

Genetic selection in the dairy industry has been relatively straightforward, with primary selection pressure based on milk production. In the beef industry, genetic merit is more difficult to define. However, defining genetic merit in an industry that produces meat is not impossible, and great progress has been made in both the turkey and pork industries, in the rates of weight gain, in feed conversion, and in carcass quality, using AI. Although the end products of the beef, turkey and pork industries are similar, there are significant similarities in the in the housing and handling of animals between the dairy, poultry and swine industries (animals are confined and/or handled on a regular often daily basis) that facilitate using AI, which is not often the case in the beef industry. It is likely that the inability to observe and handle the animals often in the beef industry is one of if not the major limitation to incorporating AI into the reproductive structure of the industry.

Advances in both the efficiency to synchronize and in reducing the costs to synchronize ovulation in cattle, may permit the economical use of AI in the beef industry.

This may be particularly true with recent marketing programs (Certified Angus Beef), animal identification systems, and new AI products such as sex-sorted semen. These advances in semen and synchronizing technologies coupled with new marketing requirements may not only make the use AI economical, it may have other benefits as well, that may make it the preferred method for inseminating at least some beef cows, in some situations and for some producers.

Current Technologies

Any new technology must provide an economical positive return on the investment, for it to be utilized by an industry. These technologies must either improve production efficiency or product quality or help meet mandated requirements. In the beef industry, implementing AI offers opportunities in increasing the genetic selection of the sires used to inseminate the females in a herd, as well as opportunities to sell genetics through semen sales. In addition, making specific sire-dam matches may be useful in directing the overall genetics of a herd, but can also be important when developing an overall identification system for individual animals. Although there are many new technologies that have been recently developed to improve male oriented biotechnologies within the AI industry, I will discuss 4 primary advances that can have an impact in male oriented technologies in the beef industry. These include improving:

1) Sire and semen quality
2) Cryopreservation of spermatozoa
3) Sex-sorting spermatozoa
4) Timing of insemination

Sire and semen Quality

Breeding Soundness of Sires

Even if a bull has superior genetics for growth rate, feed conversion, carcass quality, etc., if the cows he services do not become pregnant, none of those beneficial traits will be observed for the herd owner, and financial results will be catastrophic. Even a bull possessing lower genetic merit, but produces highly fertile spermatozoa can result in financial benefit for the owner. However, combining genetic merit traits with high fertility will result in the highest financial returns. The Breeding Soundness Exam (BSE) provides the best avenue to detect bulls that are likely to have fertility problems for bulls used in natural service. However, it should be noted that while BSEs can detect physical problems that might limit the capabilities a bull may have to service cows, scrotal circumference can predict the number of spermatozoa a bull can produce (DeJarnette and Marshall, 2007), and current semen analyses can evaluate the number of sperm in an ejaculate as well as the percentage of motile spermatozoa in the ejaculate (and possibly the percentage of viable spermatozoa, if that assay is conducted), these assays do not accurately determine the fertilizing potential of those spermatozoa (Amann, 1989, 2005; Amann and Hammerstedt, 1993; Mocé and Graham, 2007).

Improving Semen Quality

The lack of correlation between the results of laboratory semen analyses and fertility has been reviewed by others (Graham et al., 1980; Amann, 1989, 2005; Amann and Hammerstedt, 1993; Mocé and Graham, 2007). However, the inability of a sperm laboratory assay to evaluate fertility boils down to the fact that a spermatozoon must possess many traits, including every trait that researchers have tried to correlate with fertility (motility, proper morphology, properly condensed DNA, an intact acrosome, a complete complement of proteins that permit the sperm to bind to and then penetrate the oocyte, etc.), and the assay being used assesses only one or perhaps a few of those traits (Amann, 1989, 2005; Mocé and Graham, 2007). Certainly, however, a bull whose semen does not possess 'enough' of the trait we are examining (possesses very few motile sperm for instance), should be eliminated from the breeding population, as most sperm lacking a particular trait does correlate with reduced fertility (Amann, 1989, 2005; Amann and Hammerstedt, 1993; we cannot identify highly fertile semen samples, but we can identify low fertility semen samples).

New laboratory assays are being developed that help identify semen samples, and potential sires that should be eliminated from the population. Several of these assays, such as determining sperm viability and concentration by flow cytometry or the nucleoCounter were used only in laboratory settings twenty years ago, but as the price of the equipment to conduct these assays have dropped to reasonable costs, many semen producing organizations are utilizing these assays routinely (Christensen, 2002), and if reasonable selection pressures are used, semen quality is improved.

In the past most studies comparing results of laboratory semen analyses with semen or sire fertility have made correlations between the two. However, perhaps a more effective way to utilize these data is to determine which samples or bulls exhibit the poorest quality of the trait being investigated and cull them. Parrish et al. (2006) reported bulls that exhibited percentages of morphologically normal cells that were less than 1 standard deviation from the norm, could be correctly categorized as exhibiting reduced fertility with >90% accuracy. Statistically, data comprising the population below 1 standard deviation of the mean comprises approximately 15% of the data. Since many of the spermatozoal traits we evaluate in the laboratory assays are correlated with each other (Kirk et al., 2005; i.e. sperm motility is correlated with sperm viability, as non-viable sperm cannot swim), it may make sense that by culling the lowest 15% bulls, based on their semen analysis, that overall bull fertility can be improved over time.

Cryopreservation of Spermatozoa

With the report that glycerol was an effective cryoprotectant in 1949 (Polge et al.) efficient methods have been developed to cryopreserve bull spermatozoa. However, differences occur in spermatozoal biochemistry that enables spermatozoa from different species and sires within a species to cryopreserve more efficiently than others. Because of species differences, bull spermatozoa survive cryopreservation much better than do turkey, chicken or pig spermatozoa. Even within cattle, the cryosurvival rates of spermatozoa from different bulls can vary widely. Diary bulls being used in the AI industry possess the narrowest range in spermatozoal cryosurvival rates. This is due partly to their selection of only a few bulls from the many bulls that initially enter the

stud, but whose sperm failed to survive cryosurvival, and to the continued selection of genes for spermatozoal 'freezability', by always producing the next generation of bulls from frozen semen. Because of the selection process, most dairy bulls produce spermatozoa that cryopreserve reasonably well. Since there has been little selection pressure for spermatozoal cryosurvival in the beef industry, it is likely that not all bulls will produce spermatozoa that will cryopreserve well. However, extrapolating from other species that have sperm that cryopreserve reasonably well, one should expect spermatozoa from approximately one quarter of the beef bulls to freeze well, from approximately one half of the bulls to freeze reasonably well, and from one quarter to freeze poorly.

The dairy industry is organized such that only about 10% of the bulls that enter a bull stud possess sufficient genetic merit or have sperm that maintain sufficient fertility after cryopreservation, to become marketed proven sires (DeJarnette and Marshall, 2007). Because of the selection for sperm cryosurvival, by using frozen semen, and the selection processes within the bull stud, most bull studs use one specific cryo-diluent and one method of cryopreserving the spermatozoa, and have sufficient numbers of bulls for which their particular system works well enough and there has been limited effort made to develop new diluents or to customize diluents to a particular sire. This is not the case in the horse industry, where little selection pressure has been applied from the use of frozen semen for generations which results in genetics that favor spermatozoa that freeze well, or to selection from a large number of sires. In this industry a particular owner may have one or a few stallions which possess genetics that the owner wants to sell or that others wish to buy. If that stallion is part of the one quarter of stallions that produce spermatozoa that freeze well, then all is well. If, however, the stallion is not part of that population, stallion owners can try a number of different cryo-diluents, which have been developed in recent years that have different lipoprotein components, different cryoprotectants, utilize different freezing or thawing protocols etc., which may enable the sperm from that stallion to maintain sufficient fertilizing capacity after cryopreservation. This may be a similar challenge that owners of some beef bulls may face. However, new technologies in cryopreservation, including new cryo-diluents containing alternative lipid sources, from soy products (Martin et al., 2004) and sperm treatments prior to freezing, such as increasing the cholesterol content of the sperm using cyclodextrin technologies (Purdy and Graham, 2004) may help owners of specific bulls whose sperm do not cryopreserve well, using current methodologies, to preserve spermatozoa from these males.

Sex Sorting Spermatozoa

For thousands of years, mankind has sought to control the sex of offspring. However, only recently has the technology been available to separate X-bearing and Y-bearing spermatozoa into distinct populations. No differences between X-bearing and Y-bearing spermatozoa have been detected in protein content, density, swimming velocity etc. The only difference that can reliably be detected is the difference in size of the X-chromosome and the Y-chromosome of the spermatozoa, which results in X-bearing spermatozoa possessing 3.8% more DNA than Y-bearing spermatozoa. This small difference in DNA content can be detected and the sperm separated into populations of

>95% purity for enrichment of either X-bearing or Y-bearing spermatozoa, using flow cytometry (Seidel, 2003). Initial work in this area developed optimal conditions to separate the spermatozoa, including stain levels, orienting the spermatozoa properly as they pass through the laser beam, and the pressure at which the cells are forced through the machine (Schenk et al., 1999). Currently, sorting speed is the primary limitation to this technology, as only 3000-4000 live sperm of each sex can be accurately sorted each second. Although this seems like a large number, at this speed it requires about an hour to sort 10 million sperm of each sex, which is approximately the number of sperm in a conventional insemination dose (Seidel, 2003). To utilize sorted sperm more efficiently, insemination doses of sex-sorted sperm usually contain fewer (about 2 million) spermatozoa.

The sorting process compromises the spermatozoa, the dye used to stain the DNA causes a reduction in fertility, the pressure used to push the spermatozoa through the machine causes damage to the cells, the cells are physically stretched as they are released from the machine and the cells hit the collecting fluid at approximately 90 km/hr. The cells can be further compromised during the processing after sorting, centrifugation followed by cryopreservation, that is required to prepare the spermatozoa for use (Seidel, 2003b). However, even with all these insults, sex-sorted spermatozoa, when used with good management results in fertility levels only slightly lower (70-80%) than that achieved using conventionally cryopreserved spermatozoa. Because the technology works as well as it does, many bull studs now offer sex-sorted semen from several of their dairy bulls for sale. It should be pointed out, however, that spermatozoa from all bulls do not sort efficiently, whether because the cells do not stain evenly, orient properly within the flow cytometer or for some other reason. Regardless the reason, similar to cryopreservation, spermatozoa from some sires cannot be used for this technology.

The final issue about sex-sorted spermatozoa is whether it is or can be cost effective. Sexed-semen will cost more than conventional cryopreserved semen. This technology will likely be available only through large bull studs, as the flow cytometers used are expensive (~$300,000) and custom collection facilities will not likely have the resources or expertise to purchase a machine. It is estimated that a dose of sex-sorted semen will cost from $10-20 more than conventional semen, and the end user will need to determine whether the additional costs, for the initial semen and reduced fertility can be recuperated in the end. Although it seems reasonable to say, sexed-semen cannot be cost effective, there are certain situations in which it may be. For example, inseminating heifers with X-bearing sperm, to produce heifer calves may be able to reduce the cases of dystocia in a herd because heifer calves are generally smaller than bull calves, and preventing even one C-section in 100 calvings may be attractive. This might be especially true, since the youngest cattle in a herd should be genetically the best as well, and therefore, selecting these to produce the next generation of replacement animals with genetically superior X-sperm, may be cost effective. However, it is niche beef cattle markets, such as seedstock producers who may want bull calves to sell, where this technology could have its greatest impact.

Timing of insemination

As mentioned previously, beef cattle management, for many producers, is quite different than that for dairy, poultry or pig producers, in that beef cattle are often not raised in confined conditions that permit daily observations, at a minimum. One of the major hindrances to using AI in beef cattle is the difficulty in confining the animals for sufficient time periods to detect the animals in heat, in order to inseminate cryopreserved semen at the correct time. Recent improvements in methods to synchronize the ovulation in cattle, termed Ovsynch, have resulted in conception rates, using timed insemination, that are similar to AI after detected estrus (Fricke et al., 1998). A specific form of Ovsynch in which the timed AI occurs at the same time as the second GnRH injection is termed Cosynch. The advantage of Cosynch, is that one needs to handle the animals one less time. However, fertility rates using Cosynch tend to be slightly lower. The drugs used in these synchronization programs cost money, but methods have been tested to reduce the amount of hormones used in the process without reducing the efficiency of the technology, thereby reducing its cost (Fricke et al. 1998).

Summary and Conclusion

There are numerous opportunities to improve efficiency and profitability of beef production using male oriented technologies. The most simple, and least costly to implement is to increase the stringency used in culling sires after the BSE. Many of the other promising technologies can be cost prohibitive to natural service operations. However, the additional costs to implement AI in herds or perhaps part of a herd (such as only heifers) may not only be cost effective, but lead to increased profitability by increasing the genetic merit of the next generation, reducing dystocia and reducing the incidence of sexually transmitted diseases. We can, through management, produce fertility rates in our beef ranging from zero to 100 percent. However, the cost to benefit ratio to achieve a specific fertility rate is not economical at either end. Producing no calves, while input is zero the return will be even less; on the other end of the scale, achieving 100% fertility, will likely cost more money than will be realized when the calves are sold. However, recent advances in synchronizing ovulation in cattle should make the use AI with cryopreserved semen from superior bulls a distinct economical possibility for many beef producers.

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