

Practical Clinical Approaches to the Problem Breeder Female

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Abstract

Applied reproductive biotechnology, information technology, and efficient production management systems on the farm have all contributed to an increased awareness and knowledge of reproductive problems. Reproductive history is now documented and we know more than ever about individual females and their problems. Artificial Insemination (AI), embryo transfer, *in vitro* fertilization (IVF), real time ultrasound, sex-sorted sperm (Sexing Technologies, Navasota, TX 77868), and novel heat detection systems (HeatWatch®, Denver, CO 80216) are some of the tools that cattle breeders and veterinarians use to diagnose and treat problem females. Although a small percentage of infertility cases can be diagnosed and treated successfully, the majority of infertility cases in beef cattle are idiopathic in nature. Over half of the infertile females brought to my facility fall into this classification after both palpation and ultrasound examinations. In spite of all the technology available, amazingly little is known about the etiology or pathobiology of the most common form of infertility in cattle. This paper explores possible causes of idiopathic infertility and suggest a systematic method of handling bovine female infertility in a clinical setting.

Résumé

La biotechnologie de la reproduction appliquée, la technologie de l'information et des systèmes de régie de production efficaces à la ferme ont tous permis de mieux se rendre compte et de connaître les problèmes de reproduction. Les données antécédentes sur la reproduction sont bien documentées et on en connaît plus que jamais sur certaines femelles et leurs problèmes. L'insémination artificielle (IA), le transfert d'embryons, la fertilisation *in vitro*, l'échographie en temps réel, le triage du sperme selon le sexe (Sexing Technologies, Navasota, TX 77868) et les nouveaux systèmes de détection des chaleurs (HeatWatch®, Denver, CO 80216) sont quelques uns des outils que les éleveurs de bovins et les médecins vétérinaires ont à leur disposition pour diagnostiquer et traiter les femelles à problèmes. Bien que l'infertilité puisse être détectée et traitée avec succès dans quelques cas, la majorité des cas d'infertilité chez

les bovins de boucherie sont idiopathiques. Plus de la moitié des femelles infertiles admises à ma clinique se retrouvent dans cette catégorie après un examen avec la palpation et les ultrasons. En dépit de toutes les technologies disponibles, de façon surprenante on en connaît bien peu sur l'étiologie et la pathobiologie des causes les plus communes d'infertilité chez les bovins. Cet article décrit les causes possibles d'infertilité idiopathique et suggère une méthode systématique pour gérer l'infertilité des bovins femelles dans le cadre d'une clinique.

Introduction

Some females presented for failure to conceive have a problem that can be diagnosed and treated. The most common maladies include cystic ovarian disease (COD), endometritis, pyometra, mucometria, adhesions and clinical anestrous. Statistically about 30% of these respond favorably to treatment and afflicted females subsequently become pregnant. The remaining 70% are presented for diagnosis and treatment, usually after a prolonged dry period in which the female has become obese. The overweight cow seems to respond poorly to veterinary intervention. However, the largest class of bovine reproductive failure is idiopathic in nature. In these cases palpation, ultrasound exam and therapeutic flushing of the uterus reveal no pathology. There is nothing to treat. These females typically have a history of cycling at normal intervals, i.e., 18 to 23 days, but fail to conceive to known fertile sires or to frozen semen.

Classification of Infertility

There are numerous ways to classify infertility. One is based solely on the timing of ovulation, i.e., pre-ovulatory vs. post-ovulatory infertility. In other words, is the oocyte being ovulated a viable one, or are there downstream reproductive problems with the female? That question was answered in 1991 with the birth of a number of IVF calves produced from oocytes harvested from clinically infertile multiparous beef females.⁹ It became evident after most problem donors enrolled in the infertility clinic produced calves through *in vitro* production

(IVP) systems that most problem females, regardless of their history and etiology, were ovulating a healthy oocyte. As long as a cow had a reasonable number of small follicles on her ovaries and was cycling she was a candidate for ultrasound ovum pick up (OPU)³ and IVF. That told us that the oocytes in general were healthy, but there were other problems causing the infertility.

It is largely agreed that most idiopathic infertility can be classified as post-ovulatory. However, from the point of ovulation things get a little less clear. Some embryo transfer donor females will produce viable embryos after superovulation, AI, and embryo collection, but their system fails to recognize pregnancy.¹ In other words, they will produce viable embryos, but won't carry one of their own to term. Other cows will maintain pregnancy for a short while before embryonic death, but some will cycle at normal intervals even with a viable embryo in their uterus. We classify the females that carry the conceptus through one or more cycles as "embryo producing short term carriers". Those that produce viable embryos but won't hold through the first cycle period are called "embryo producing non-carriers".

Females that cycle normally and fail to get pregnant, and that produce unfertilized ova after superovulation, AI, and embryo collection are classified as "idiopathics". Conventional breeding (bull and AI) and treatment protocols repeatedly fail in this class of female. They have no apparent problems diagnosed by palpation and or ultrasound. Either sperm is not being transported to the oviduct where fertilization occurs, or the environment in the uterus or oviduct is severely detrimental to sperm. Unfortunately, this is the largest single classification of infertility presented to us. They are essentially untreatable by conventional veterinary therapy.

There is the rare classification of female that will allow fertilization to occur, but when her embryos are collected at day 7 post-estrus most are arrested at the four to 16 cell stage. Those are called degenerated (fertilized but dead) ova. We assume that sperm is capacitated, oocytes are fertilized, and that the embryo cleaves normally in the oviduct of these females. However, once the embryo is dumped into the uterus it ceases to cleave and dies.

Potential Pathophysiology of Idiopathic Infertility

Sperm transport mechanisms in the female could be a major cause of idiopathic infertility.^{4,6-8} Kraemer *et al* did a nice experiment that showed sperm transport in the cow is not reduced significantly by superovulation.⁵ He showed that superovulated females had about the same number of sperm in the isthmus of their oviduct at the time of ovulation than did non-stimulated

controls. However, this experiment was only a snapshot in time and not a dynamic study of sperm transport over time. His experiment was like standing on an overpass over the interstate and taking a single picture of the traffic below and counting cars in the field of view as compared to taking videotape over a 12 or 24 hour period and then counting the total number of cars that passed. As important as sperm transport dynamics are in the bovine and as important as infertility is to the industry, someone needs to design an experiment to study that concept. Do sperm continually pass from the uterus into the oviduct over a period of time, or are only a few allowed to pass and do the fertilizing? Are sperm transport dynamics different in idiopathics as compared to normal controls?

If sperm transport mechanisms isn't the problem with idiopathics, then what about environmental conditions in the uterus or oviduct? Capacitation is necessary for sperm to be able to fertilize. Is sperm being capacitated in idiopathics? If it is and ova are still not being fertilized in the oviduct, then is there some toxic substance being produced by these idiopathics, and is its origin the uterus or the oviduct? The industry needs to know this basic information since this is the most common form of infertility.

Systematic Approach to Diagnosis/Treatment

My practice career has been 100% devoted to reproduction in beef and dairy cattle for the past 26 years, with more emphasis on beef the last 15 years. Most of the practice is devoted to embryo transfer, but we also house problem females for diagnosis and treatment. When a reproductive problem female is presented the first thing on the list is to get a complete history. We look for possible management induced errors before we even examine the female since she may not have a problem at all. Has the female been with a fertile male known to have successfully bred other females during the same time period? If the female has been artificially inseminated is the semen known to have settled other females during the same time period? If AI has been attempted what kind of track record has the technician had? We also examine the thawing procedures, and more importantly examine the technician's semen handling procedures. We also like to evaluate the frozen semen being used to inseminate the female. Also, heat detection protocol on the farm needs to be discussed. Ownership and management make a tremendous difference on our approach to diagnosing a problem breeder. With some managers we don't even ask the above questions. We know everything is in order, and that the female being presented has a problem, not the owner/manager.

Once we're satisfied the animal has a problem the first question asked concerns parity. The female is clas-

sified into one of two categories; 1) parous or 2) nulliparous. The second question is the age of the female. If the female is a virgin heifer, and is already three years old, statistically the odds are against successfully treating even a common malady and then achieving pregnancy. By the age of three years, most heifers have cycled dozens of times since puberty and failed to become pregnant. Even if a cystic ovary is discovered upon examination treatment may be successful for the cyst, but fail to achieve the goal of pregnancy afterwards. It's possible that this type of female could produce viable embryos, but is this type of infertility acquired or genetic in nature? With virgin heifers one never knows, and transferring embryos from her may be passing the problem to the next generation. A frank discussion with the owner is appropriate before recommending superovulation and embryo recovery or IVF as an alternative means to genetic resuscitation.

With parous females we can assume that most infertility is acquired and not inherent. If the cow in question is over 10 to 12 years of age we perform a reproductive exam and try to fix any diagnosed problem. Then we try to prepare her for superovulation and embryo collection instead of trying to get her pregnant since age alone could be why she's failing to conceive and/or hold a conceptus. If she's not worth being classified as an embryo donor, we tend to discourage diagnosis and treatment of a cow that age. The return on investment is a poor risk.

If nulliparous females are under three years of age, and we diagnose a treatable problem, we attempt therapy and then suggest putting her with a known fertile male or AI with good quality semen. If traditional breeding fails we then have the discussion of heritability with the client. If he's invested thousands of dollars in this young female, we suggest trying to return her for a refund or heifer of equal value. If those efforts fail and he insists that we move forward with alternative therapies we suggest a single embryo flush as a diagnostic tool. Less than 20% (23 of 126) of the time, we will collect a viable embryo from a problem virgin heifer. 80% of the time collected ova will be unfertilized. Sometimes we do the single egg collection twice before giving up as only 60% of the time we actually collect an ova at all. If we do collect a viable embryo then we can assume that she is not capable of recognizing and or maintaining a pregnancy. That creates another discussion with the owner. We can superovulate her and use her as an embryo donor until we get some progeny from her, or he can cease to attempt therapy and cut his losses. By choosing to superovulate her he could be passing a bad gene to the next generation of progeny.

If a single egg collection on a problem virgin heifer produces an unfertilized ovum, we suggest using her as a recipient for another female's embryo as a last resort.

If she isn't transporting sperm nor has a non-supporting environment for sperm, she may be capable of recognizing pregnancy herself and carrying the resulting fetus to term. If we drop a viable embryo into her uterus (we give at least two chances with grade 1 embryos) and she becomes pregnant and goes term, about 40% of those females gain the ability to conceive on their own after calving and lactating. It's as if the pregnancy and subsequent lactation "re-boot" their reproductive computers. Those that do conceive naturally post-calving go on to be reproductively normal afterwards. The larger majority, however, continue to be problematic. By then the owners usually give up and take them to slaughter.

With young to middle-aged problem parous females we take a slightly different approach to managing infertility. The assumption is that the infertility is acquired and not inherited. If we diagnose a treatable problem, we try to correct it and breed to a bull or AI with good quality semen, (preferably a bull). If conventional treatment fails we treat them like idiopathics from that point forward. We usually skip the single egg flush and go directly to superovulation and embryo collection for production. If the female isn't worth the expense of embryo collection we recommend culling or putting her with a bull. We examine every few months until pregnant or bust. It's bust the large majority of the time.

If the cow has some relative worth we superovulate and attempt to collect embryos from her. About 20% of the time we recover viable embryos from this class of female. As long as they keep producing embryos we keep them on the payroll. For those that don't produce viable embryos we revert to transferring embryos from another donor into them. Some will get bred (about 40%) and others won't. Again, as with virgin heifers, of those that get pregnant and go to term and lactate, about half will conceive on their own afterwards. The "re-booting effect" seems to work equally as well on virgin heifers and cows.

That leaves us with a class of problem parous females that reproductively appear to have a bleak future on their own. They have proven unsuccessful with our treatment strategies, haven't responded to superovulation, and haven't conceived to a transferred embryo. Currently, the best options for those females are IVF or cloning. There are pros and cons for both.

IVF as a Reproduction Option

As mentioned previously IVF tends to produce embryos from almost any class of subfertile or infertile female. Since not all sires produce frozen semen that is successful in IVF programs, the owner may have to settle for a different bull, but the *in vitro* production systems are pretty efficient at producing embryos from otherwise problem breeders. Some of the drawbacks to IVF

are that the donor animal needs to be shipped to a remote facility for management where weekly or bi-weekly OPU sessions take place. *In vitro*-produced embryos do not perform well after freezing and transfer. They should be transferred fresh, which means the embryo transfer center will be providing recipients for the embryos produced. That increases the bottom line from an economic perspective. Also, there are only a few commercial bovine IVF labs operating in the US today.^{a,b} IVF expenses are a notch higher than traditional embryo transfer, so the client must weigh all the factors before making the decision to go that route.

Cloning as a Reproductive Tool

Cloning by somatic cell nuclear transfer is now another option for the dead-end problem breeder, but the cow must be very valuable as the cost for one copy is well over \$10,000. For the super valuable females with acquired infertility, it makes sense to biopsy and clone to get several copies. Once the clones have hit puberty the owner's options are multiplied by the number of copies produced. At that point, he can put them in embryo transfer and mate to more modern sires, but, more importantly, the clones have an entire reproductive life ahead of them. That's about as good a result as can be expected from the original cow that is basically out of options. Also, once the donor has been gene banked and before the clones hit puberty, she can be enrolled into an IVF facility to make a few calves during the three year waiting period (1 year from biopsy to calf-on-ground, 1 more year until puberty, and 1 more year for a calf-at-side) for production from the cloning process.

Conclusion

There are many causes of reproductive failure in the bovine female. Diagnoses and treatments are often unsuccessful. In order to assist cattle breeders with making sound decisions on problem breeders it behooves the veterinary practitioner to take a systematic approach

to diagnosing and treating them in the most practical least cost manner. A good history can rule out management as a probable cause, but a thorough exam and the tools to diagnose and treat are essential. The most important approach to the problem breeder is knowing all the options, and in what order they should be employed. Recommending culling without treatment is often the most valuable advice a practitioner can give his client. However, some clients need to know that the practitioner has exhausted all practical options, including referral, before he willfully accepts culling as the proper option.

Endnotes

^a OvaGenix, Bryan, Texas 77805

^b Trans Ova Genetics, Sioux Center, IA 51250

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