APPLYING REPRODUCTIVE TECHNOLOGIES IN PRACTICE

William L. Flowers
Department of Animal Science
North Carolina State University
220-B Polk, Raleigh, North Carolina, 27695-7621
E-mail: william_flowers@ncsu.edu

ABSTRACT

One of the most important aspects of applying reproductive technologies in practice is to determine whether they improve performance under farm conditions. What this really entails is conducting an "on-farm" experiment. Three of the most important aspects to consider when conducting "on-farm" experiments are the length of the treatment period; inclusion of a untreated contemporary group; and evaluating adequate numbers of animals. The length of the treatment period is determined by the specific physiological aspect that the new technology is supposed to influence. For example, technologies designed to increase the quantity and quality of sperm produced by boars need to be applied for at least 7 weeks because this is the normal length of time associated with spermatogenesis in swine. In contrast, technologies affecting the timing of estrus and ovulation only need to be administered for weeks or days because these processes occur over a shorter period of time. Inclusion of temporary, untreated groups is necessary to avoid confounding of results. Confounding refers to a situation in which two or more factors are altered at the same time which makes identifying the one that created a response virtually impossible. Finally, adequate numbers of animals have to be tested. This assists producers in determining whether any advantages in performance can be confidently attributed to the new technology or whether they are simply due to chance.

INTRODUCTION

Implementation of new reproductive technologies in swine production involves both basic and applied studies. Basic studies are typically done with small numbers of animals under very controlled conditions. They demonstrate that the technology being investigated influences biological mechanisms. For example, number of pigs born alive is a function of ovulation rate, fertilization rate and embryonic survival. Consequently, any technology designed to increase litter size has to improve one or more of these reproductive events in order to be effective. Basic studies essentially establish "why" or "how" new technologies work.

In contrast, applied studies are conducted with large numbers of animals under what is referred to as "field" or "farm" conditions. They provide critical information in terms of the range in responses that can be expected. For example, it has been well documented that parity structure, disease status, and season of the year all significantly affect how herds respond to a number of different treatments and, as a result, it is not unusual to see small or no responses on some farms, yet robust improvements on others. Applied studies help identify "when" and "where" on swine operations new reproductive technologies should be implemented.

If a producer reads about a new technology in a trade magazine or hears about one at a conference and decides to try and implement it on his or her operation, then he or she is conducting an applied experiment. In fact, most of the applied studies conducted with pigs probably are performed by producers who are interested in determining whether a new management practice, feed additive, or the like can improve their herd's reproductive performance. There is no single "right way" to conduct an "on-farm" experiment. However, there are several things that are common to all good studies. These include:

a) treatment periods long enough to allow them to work biologically;
b) contemporary groups that reflect normal, untreated management practices; and
c) sufficient numbers of animals to accurately determine differences.

Careful attention to these three details will insure that the information producers collect and the decisions derived from it are accurate, unbiased, and apply to their farms.

LENGTH OF TREATMENT PERIODS

The length of the treatment period really depends upon the reproductive process that is trying to be improved, which requires some knowledge of the reproductive physiology of both boars and sows. Figures 1 and 2 summarize key reproductive events associated with production of spermatozoa in boars and live pigs in sows, respectively.

Figure 1. Schematic representation of sperm production in boars.

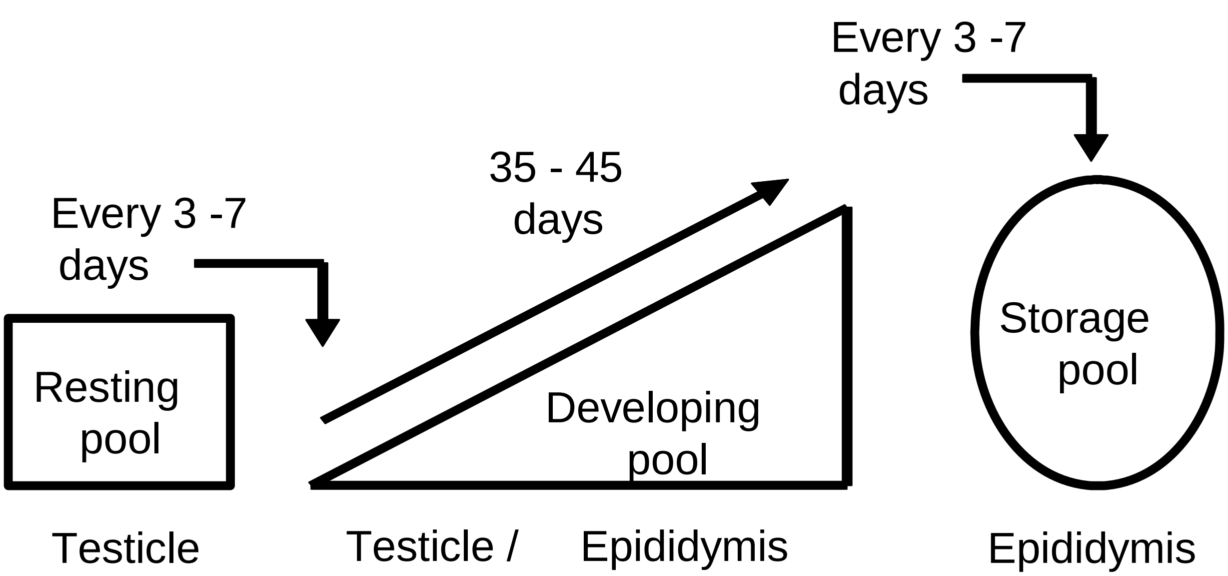
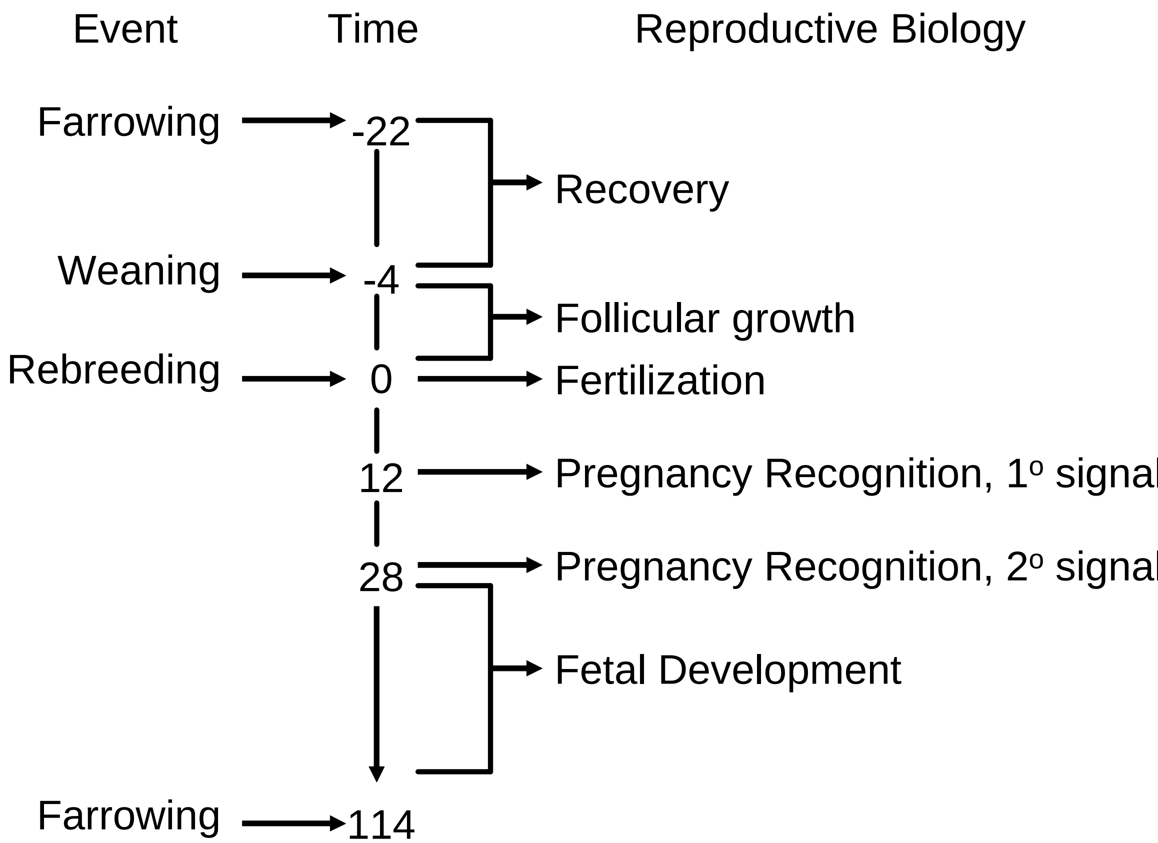


Figure 2. Schematic representation of key reproductive events in sows.



Production of viable, fertile sperm cells is referred to as spermatogenesis. In mature boars, every three to seven days, there is a new group of immature sperm cells that leave the resting pool located in the testicle and begin to mature or develop. While they are undergoing development, each sperm cell that left the resting pool divides repeatedly forming many new copies of itself. It takes between 35 and 45 days from the time sperm cells leave the resting pool until they are mature and capable of fertilization. Once the process is complete, mature spermatozoa are stored in the tail of the epididymis until they are released during ejaculation. It makes physiological sense that if a new group of sperm cells starts to mature every 3 to 7 days, then a new group of mature sperm cells enters the tail of the epididymis at the same frequency, 35 to 45 days later.

This information is important when applying reproductive technologies that are designed to increase the quality and/or quantity of sperm produced from boars. The three primary ways to increase the quantity of sperm cells boars produce are to increase the frequency at which new sperm cells begin to develop; decrease the length of time required for maturation; or increase the number of copies sperm cells make of themselves during development. Regardless of which of these are affected, it probably takes at least 6 to 7 weeks (35 days) after a treatment has been applied to observe any noticeable changes. This length of time is consistent with the normal maturation period of sperm cells. In other words, if one assumes that the application of the new technology coincided with a new group of spermatozoa entering the developing pool, then it would be 6 to 7 weeks before this group of spermatozoa would be mature and reach the tail of the epididymis. These would be the first sperm cells that were exposed to the treatment throughout their entire developmental period.

In this time line, the day that the sow is rebred after weaning is considered to be Day 0 or the onset of pregnancy. Events that occur before and after breeding are designated with negative and positive numbers, respectively, in accordance with how long they occur either before or after the onset of pregnancy. The time line begins with the first day of lactation, which happens to correspond with the birth of the litter from the previous pregnancy and ends with birth of the litter from the current pregnancy. In essence, as soon as one pregnancy ends, physiological processes are initiated that begin to prepare a sow for her next one.

After farrowing, the reproductive system of sows requires time to recover from the previous pregnancy. The three most important organs involved with this process are the ovaries, brain, and uterus. The ovaries contain follicles, which grow in response to two hormones produced by the brain, luteinizing hormone (LH) and follicle stimulating hormone (FSH). These follicles will eventually ovulate after weaning and release their eggs to be fertilized during rebreeding. The ovaries recover very quickly and their follicles are capable of resuming normal growth, if properly stimulated, within a few hours after farrowing. The brain normally does not acquire the ability to produce sufficient levels of LH and FSH to support the final stages of follicular growth and ovulation until 10 to 12 days after farrowing. The uterus is where the majority of embryonic and all of fetal development occur during pregnancy. Its recovery also contains two phases, but requires between 14 and 16 days under normal conditions.

From a physiological perspective, fertilization requires that sufficient numbers of fertile spermatozoa be present in the oviduct several hours prior to ovulation. Consequently, from a management perspective, things such as semen quality, detection of estrus, and the technical competence of breeding technicians all play important roles in the relative success or failure of fertilization. However, provided that these are all done reasonably well, fertilization rates in pigs are usually very high, often exceeding 90%. Around day 12 of pregnancy, embryos begin to produce estrogens. If sows receive the first signal by day 12, then pregnancy is maintained. The embryos continue their development and actually begin to attach to the uterus, which is commonly referred to as implantation. Sometime after day 17 and before day 28 of pregnancy, the developing embryos initiate a second period of estrogen production. It is thought that this second pregnancy signal is associated with the development of the fetal portion of the placenta.

After day 30, when implantation is complete, the developing embryos begin to resemble live pigs so they are referred to as fetuses. For the remainder of pregnancy fetuses grow and develop and should reach a physiological state in which they are capable of surviving outside the uterus, around 114 days.

In comparison, the sow's reproductive cycle is more complex than the boar's. Thus, recommendations with regards to how long a treatment or technology should be applied vary considerably and really should be based on how the technology is thought to affect the sow's reproductive physiology. This is one of the main reasons why basic research is so important and is usually conducted before applied studies. Without going into exhaustive explanations, some general recommendations for the length of time treatments need to be applied to sows in order to have a biological impact are outlined in Table 1. These should be viewed as recommendations and it is important to recognize that depending on how the treatment or technology affects the sow, then there could be significant deviations to these suggestions. For example, in theory, it is possible for a single injection of a pharmaceutical to stimulate a sow for several days, weeks, or months depending on how quickly it is cleared from the sow's body. A treatment or technology with these properties might only need to be applied once, whereas one that basically does the same thing but has a shorter biological half-life might need to be applied several times. Therefore, the term "effective period" is used in Table 1. This term refers to how long the treatment or technology would need to remain active in terms of affecting the sow regardless of how many times it needed to be actually administered to the sow.

A tendency that is common to farm studies is to apply the new technology to every animal on the farm at a given point in time; collect data for a specified period of time; and then compare the performance of the herd "before" and "after" the technology was implemented. This typically is very easy to implement, because all the animals in the herd either do or don't receive the new technology at the same time. This type of approach is valid if all the animals and everything about the production environment (feed, labor, temperature, disease status) remained constant for the duration of the evaluation period. Unfortunately, this does not happen very often, if ever, in practice. What usually happens in studies conducted in this manner is that changes over time in the production environment or the animals themselves that are unrelated to the treatment or technology can bias the outcome of the data. Animal and veterinary scientists call this "confounding". When two things are confounded, it means that they were changed or altered at the same time and there is no way of knowing if a response is to the result of the changes in one or both of them.

A good example of confounding is shown in Figure 3. This was a study that was conducted on a commercial swine farm to investigate the use of oxytocin as a pre-breeding treatment. The study was conducted a number of years ago when A.I. was first being implemented in the U.S. on a large scale. The rationale was that oxytocin stimulates uterine contractions and is a normal constituent of semen. Consequently, by giving it to the sow prior to breeding, it should facilitate uterine contractions and improve the transport of spermatozoa in the female reproductive tract during insemination, which in turn, should improve fertility. The study was actually conducted in a manner in which sows within parity groups were selected to receive or not to receive oxytocin just prior to insemination. In other words, a contemporary, untreated control group was included. These results are shown in Panel A. Panel B contains the same data, but it only includes information from the untreated group during the first half of the study and the treated group during the second half of the study – a situation that is analogous to beginning treatment of the entire herd at a single point in time and then making a "before" and "after" comparison.

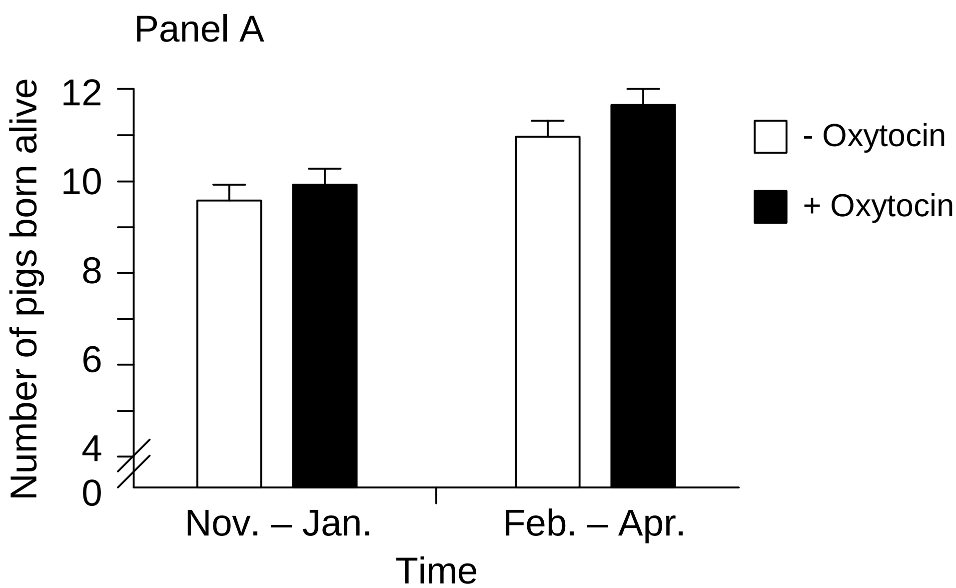
Table 1. **General recommendations for the effective period of selected reproductive technologies for sows.**

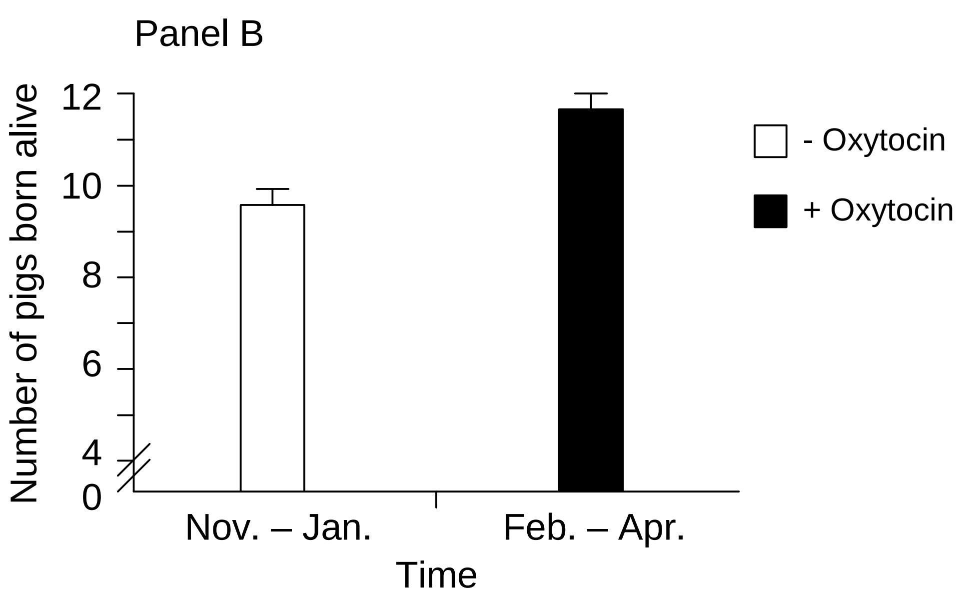
Goal of Technology	Effective Period	Rationale
Synchronization of Estrus	Between Weaning and Rebreeding	Final stages of follicular growth occur during this period
Synchronization of Ovulation	Between weaning and rebreeding but closer to rebreeding	Final stages of follicular growth occur during this period and ovulation occurs after estrus
Increase Fertilization	Rebreeding	Fertilization occurs over a 6 to 12 hour period during estrus
Increase litter size by affecting ovulation rate	Day 1 of lactation through rebreeding	Follicles ovulated after weaning begin to grow after farrowing
Increase litter size by directly affecting embryos	Rebreeding though day 30 of pregnancy	Critical aspects of embryonic development occur during the first 30 days
Increase litter size by directly affecting fetal development	Day 30 of pregnancy through farrowing	Fetal development begins after implantation and continues until birth
Increase litter size by directly affecting uterine function	Day 1 of lactation through the next farrowing	Uterine recovery begins during lactation and uterine function is necessary throughout all of pregnancy

INCLUSION OF CONTEMPORARY GROUPS OF UNTREATED ANIMALS

The results from the study with a contemporary untreated control clearly show that there was no difference between the two treatments. In other words, oxytocin pre-treatment did not affect litter size on this farm. However, the overall litter size did increase in both treatments over time. It was higher for sows bred between February and April compared with sows bred between November and January (Panel A). It is important to notice what the data would have looked like if the study was conducted in such a way that all the sows in the herd began receiving oxytocin in February and their performance was compared to sows receiving no treatment between November through January. Based on these data shown in Panel B, the conclusion would have been that oxytocin pre-treatment had a significant effect on number of pigs born alive. In fact the difference would appear to be almost 2 pigs per litter. Obviously, something improved on a herd-wide basis with regards to the number of pigs born alive between the November/January and February/April. (In this particular case, it was due to the fact that the A.I. technicians became more comfortable with the insemination process, etc.). By including a contemporary control group of untreated animals, the conclusion from the study was that technology being evaluated, in this case, did not have an effect on reproductive performance. In contrast, without this group, the conclusion from the study would have been that the reproductive technology did increase litter size.

Figure 3. **Effect of oxytocin pre-treatment on litter in swine. Panel A illustrates data analyzed with an untreated, contemporary control (correct design). Panel B illustrates how data would appear with a "before" and "after" comparison (incorrect design).**





ADEQUATE NUMBERS OF ANIMALS

To determine whether new technologies should be adopted, it is important that they be tested on enough animals so that producers have confidence that any improvements seen are due to the technology and not other unrelated factors. As mentioned previously, parity structure, disease status, and season all affect reproductive performance and as a result farrowing rates and litter sizes often change over time within a herd. When a new technology is used, it is important for producers to know whether the advantages seen in the treated animals are due to the new technology or to unrelated, normal fluctuations that are present in every herd. There are three questions that producers need to consider when determining how many animals need to be tested in order to determine whether a new technology is working. These are as follows:

1. How large of an improvement does the new technology need to produce in order for it to be used routinely;
2. How much normal variation is present in my herd; and
3. How much confidence does one want in the results?

The answer to the first question is herd specific. Factors such as the cost of the new technology and the current level of performance in the herd are important considerations. Typically, there is a positive relationship between the cost of the technology and the level of improvement in performance that needs to occur. Technologies that are inexpensive probably do not need to produce as much improvement as those that are expensive. Indirect costs such as the labor required to implement a new technology should be considered as well. If additional labor is required to use something, then the actual cost to the farm will be higher than just the cost of the technology. Conversely, if a new technology reduces labor, then a higher initial cost might be justified.

The current level of performance of the herd also needs to be considered. Herds with low numbers have more room, or opportunities for increasing productivity compared with high-performing herds. The average litter size within a herd is a good example. It is unlikely that a herd with an average born alive of 13 piglets would be able to increase litter size by 1 pig per litter in response to a new technology. This is due to the fact that the biological limit for most modern maternal lines of sows is probably between 13 and 14 piglets. In contrast, an increase of this magnitude would be more likely in a herd that only averages 9 pigs born alive.

Knowledge of the normal amount of variation in a herd is important for the reasons discussed previously – producers need to know to what degree things such as parity structure and season affect performance in order to be confident that results they obtain from a new technology are in fact due to the new technology and not due to other unrelated changes inherent to the herd. The most common estimate of variation used is called the standard deviation. In statistical theory, the average plus or minus 1 standard deviation should encompass about two-thirds of the entire herd and the average plus or minus 2 standard deviations should encompass over 90% of the entire herd. For example, the standard deviation for litter size in most swine herds is around 2.0 pigs. For a herd with an average of 10 piglets born alive, on average, two-thirds of the litters farrowed would contain between 8 and 12 piglets and over 90% would contain between 6 and 14 piglets.

In general, there is an inverse relationship between the normal variation in a herd (as estimated by the standard deviation) and the level of management. In other words, as management expertise increases, the standard deviations for reproductive measures decrease.

The degree of confidence in the results deals with minimizing mistakes. There are two basic kinds of mistakes. One is the situation in which the new technology does improve performance, but the results from the study indicate that it doesn't. The second is the exact opposite – the new technology really doesn't improve performance, but the results from the study indicate that it does. Obviously, both are bad from a practical perspective. No one wants to use something thinking that it works when it doesn't. This simply wastes money. Conversely, if something really does work, then most producers would want to incorporate it into their management programs. These two types of mistake can occur for a number of reasons and from a statistical perspective the best way to minimize their occurrence is to test a sufficient number of animals. In general, there is a positive relationship between the level of confidence and the numbers of animals that need to be used when evaluating a new technology – producers that want more confidence in the results of their evaluations need to use more animals.

Relationships among normal herd variation, numbers of animals per treatment and the relative advantage that a new technology would need to produce in litter size are shown in Table 2. These calculations are based on a confidence level of 90%. One way to use this information is as follows:

1. Select the estimate of normal herd variation (standard deviation) in litter size that is closest to the herd in which the new technology is going to be applied;
2. Within that row, find the advantage in litter size that would make the new technology cost effective with the herd; and
3. Within that column, find the numbers of animals required per treatment.

For example, let's assume that a new technology becomes available and a herd with a standard deviation of 2.0 pigs wants to determine if it is something that they should incorporate into their normal management program. Let's also assume that based on their current level of production and production costs that the technology would have to increase litter size by 0.5 pigs. Table 2 indicates that they would have to collect litter size data from 200 untreated animals and 200 animals that received the new technology in order to determine whether it was effective at increasing litter size. After collecting the data from all 400 animals, if the average litter size from the animals treated with the new technology was 0.5 pigs or higher than the untreated animals, then the farm could be reasonably assured (90%) that the observed increase in litter size could be attributed to the new technology.

Table 2. Estimates of the relative advantage in litter size required of new technologies in order for them to be statistically significant (based on 90% confidence levels[1]).

Herd Variation (Standard Deviation)	Numbers of Animals per Treatment			
	50	100	200	500
1.0	0.5 pigs	0.4 pigs	0.3 pigs	0.2 pigs
2.0	1.0 pigs	0.7 pigs	0.5 pigs	0.3 pigs
3.0	1.5 pigs	1.1 pigs	0.8 pigs	0.5 pigs

[1]Power = 0.90 and $\alpha = 0.10$.

CONCLUSIONS

One of the most important and first steps for the successful application of new reproductive technologies is to evaluate their effectiveness on the farms in which they will be used. This involves conducting a "field study" or applied experiment. The length of time that they are applied to individual animals; measurement performance from a contemporary group of animals that are not treated; and monitoring an adequate number of both treated and untreated animals are necessary to evaluate whether new technologies actually improve reproductive performance.