UPDATE ON SWINE REPRODUCTION

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This update on swine reproduction will cover a broad range of topics from assisted reproductive technologies to contemporary disease concerns. It is the goal of this paper to briefly touch on the historical background of many topics and then provide an understanding of how they fit into the current practice of swine reproductive management. Emerging technologies that are not yet a part of routine swine practice, but that are destined to make a major impact in the near future, are also introduced.

I. Artificial Insemination

Artificial insemination in swine has grown from <10% of sows receiving at least one AI in 1991 to approximately 56% of litters born in 1999 having been conceived using AI (Burke 2000). In the USA within the next 5 years, it is expected that 80% of litters will be sired by AI. Currently, boar semen is used fresh within 72 hours of collection, with 5 to 6 days being the practical limit for storage of extended semen in selected extenders without observing a significant decline in fertility (Kuster and Althouse 1999). Doses of AI semen are packaged in bags, tubes or bottles and generally contain 2 to 4.5 billion total sperm cells in 75 to 80 ml of extender. Extended pig semen is stored at 16º C to 18º C. Sows and gilts are observed for signs of estrus once or twice daily with most females receiving two inseminations per service, once each day of standing estrus. Breeding saddles and other devices that allow the AI technician to inseminate more than one sow at a time are becoming increasing popular in large commercial sow units. The use of these devises also reduces technician “fatigue”, whereby sow performance decreases with number of sows inseminated.

II. Reduced sperm numbers per insemination dose

The total number of sperm cells contained in a typical dose of AI semen has steadily decreased with increased experience with AI in the USA. While it was not unusual to use 5 to 8 billion sperm per dose in the early 1990’s, 3 billion sperm per dose is commonly used today, with efforts being made to further reduce sperm numbers to between 2 to 2.5 billion to stretch boar power. As sperm numbers are reduced, the method of estimating sperm cell concentration in the raw ejaculate becomes more critical. Traditionally, photometric measurement of transmittance or absorbance has been the most popular technique for indirectly estimating concentration. However, the seminal fluid opacity varies significantly among boars and ejaculates, which may result in erroneous readings of up to 30% off the actual concentration (Woelders 1991). For this reason, CASA systems are beginning to be utilized in the industry to directly count individual sperm cells in a sample of diluted semen as well as to provide objective analyses of motility and morphology parameters.

When low sperm numbers are inseminated the site of sperm deposition becomes important and recent research interest has focused on intrauterine insemination. In contrast to the traditional intracervical insemination method, instruments and techniques are currently being developed to allow the semen to be deposited directly into the uterine body or uterine horns. One branch of this effort is focused on delivering extremely low numbers of sperm (10 to 150 million) near to the uterotubal junction using either surgical (Krueger and Rath 2000) or endoscopic techniques (Vazquez et al. 2000; Martinez et al. 2001a). At this time these strategies would only be justified if dealing with semen of extremely high genetic value, or for sex pre-selected semen. Very recently, a new catheter has been designed that allows entry into a uterine horn to within 25 cm of the uterotubal junction. Acceptable fertility was seen with insemination of 50 to 150 million sperm (Martinez et al. 2001b; Roca et al. 2002). What is not resolved is how acceptable litter sizes can result from a unilateral uterine horn sperm deposition. Commercially, several companies have developed AI catheters that are capable of being passed via manual manipulation through the cervix of the sow and depositing the semen dose into the uterine body. As a viable option, this technique allows sperm numbers to be reduced to one billion per insemination dose. Field trials have confirmed that fertility levels statistically comparable to standard AI can be maintained using this technique (Watson and Behan 2002). However, the fecundity index (farrowing rate x litter size) is usually reduced following intrauterine insemination suggesting that this technique needs further development. Interestingly, farrowing rates to intrauterine inseminations decrease with duration of the wean-to-estrus interval (K. Rozeboom, unpublished data) suggesting a possible relationship between sperm numbers deposited, time from deposition to ovulation, and sow fertility.

III. Semen Sexing

The ability to sex sperm would be of great benefit to the swine industry for the targeted production of parent boars and gilts. At this time the Beltsville Sperm Sexing Technology is the only consistently proven method for sorting swine semen (Johnson et al. 2000). However, much research is being conducted on the possibility of using immunological detection of sperm plasma membrane proteins associated with the Y-chromosome (Althouse 2000). Although piglets have been produced from sexed semen, this technology in not yet efficient enough for use even in swine genetic nucleus programs (Johnson et al. 2000).

IV. Semen Cryopreservation

The ability to use frozen-thawed semen is an important goal in swine reproduction, as it would provide for enhanced biosecurity. However, the use of frozen-thawed porcine semen is still limited due to inconsistent reproductive performance compared to natural service or fresh semen AI (Johnson 1998). New insemination strategies have been developed which result in improved farrowing rates and normal litter sizes but these protocols are highly labor intensive and require 3 to 4 inseminations per female (Martin et al. 2000). The single intrauterine insemination of frozen-thawed semen was successful when the time of ovulation was controlled (Roca et al. 2002). Taken together, these data support the suggestion that fertility to a frozen-thawed insemination will be acceptable only if performed in the immediate peri-ovulatory period. Future research will focus on preventing/minimizing cryo-injury and repair of cryo-injury prior to insemination.

V. Embryo Transfer and Related Technologies

Embryo transfer has the potential to allow global dissemination of genetic material without risk of disease spread. Since the zona pellucida appears to be completely impermeable to known pathogens, properly washing zona-intact embryos appears to eliminate the risk of disease (Pollard and Plante 1998). Typically, the donors and recipients are synchronized by using batch-weaned sows, aborting bred gilts, or using exogenous hormones (e.g. gonadotrophins), so that embryos can be transferred to a recipient that ovulated between 24 hours before to 12 hours after the donor. Embryos are surgically collected, usually 4-5 days after onset of estrus, and are then surgically transferred to recipients within a few hours. Farrowing rates and litter sizes have approached natural breeding parameters using this method, although at least 30% of the transferred embryos are expected to be lost (for detailed reviews, see Cameron et al. 1989; Hazeleger and Kemp 1999; Cameron 2001). Surgical transfer of embryos is still the method of choice for most ET programs (Garcia 2001), but ET will not become commercially viable until a reliable, user-friendly non-surgical transfer technique is developed. Several groups are making progress in this area, including the use of deep intrauterine insemination catheters (Martinez et al. 2002).

VI. Cryopreservation of porcine embryos

Cryopreservation of porcine embryos is particularly difficult compared to other food animal species. This is due in part to their high cytoplasmic lipid content which predisposes them to cell perturbation during the cooling process (Dobrinsky 2000). Vitrification procedures have been applied to pig embryos, inhibiting the formation of ice crystals that damage the cells. Vitrification could potentially make porcine embryo cryopreservation more successful in the future, especially when coupled with other additional stabilization techniques such as displacement of lipid granules, delipidation and cytoskeletal stabilization (Cameron 1998; Dobrinsky et al. 2000; Nagashima et al. 1999). Other assisted reproductive technologies that have been developed and applied to swine under research settings include gamete intra fallopian transfer (GIFT), intra-cytoplasmic sperm injection (ICSI), in vitro fertilization (IVF) and in vitro maturation (IVM). Unfortunately, these technologies are too specialized and costly to be immediately applied to the swine industry. Near future applications may include special seed-stock grandparent or possibly parent breeding programs (Althouse 2000).

VII. Marker Assisted Genetic Selection

Traditional genetic improvement programs utilizing best linear unbiased predictors (BLUP) have been largely limited to traits that can be accurately measured in large numbers of animals (Montaldo and Meza-Herrera 1998). Marker assisted genetic selection is currently being actively utilized by the swine breeding community to make more efficient progress in identifying both genotypes that are desirable and worth propagating such as the ESR gene (Rothschild 1996), as well as animals that carry undesirable qualities such as the RN gene (Le Roy et al. 2000). These technologies allow more rapid improvement in breeding programs without entering the controversial territory of "genetic modification".

VIII. Real-Time Ultrasound

Within the last 5 years, real-time ultrasound (RTU) has been increasingly utilized for the diagnosis of pregnancy in swine. Application of this technology has had a great impact on the swine industry. Older technologies, such as amplitude depth (a-mode) and Doppler instruments

have begun to fall out of favor as they are prone to greater error in terms of false-positive and false-negative error rates and are not reliable until 30+ days after mating (Almond et al. 1998). An accurate and reliable method of pregnancy diagnosis was needed in the swine industry, since under field conditions an average to good producer will only identify 50% to 70% of open sows by heat detection alone at 17 to 24 days post breeding (Almond et al. 1998). Several RTU units now on the market are lightweight, portable, and relatively inexpensive. For external trans-abdominal pregnancy diagnosis alone, 3.5 MHz sector scanners or 5 MHz linear probes are the most commonly employed, and are accurate in trained hands beginning at 22 days of gestation (Knox and Althouse 1999).

With appropriate transducers (5.0 to 7.5 MHz), real-time ultrasonic imaging can also be utilized to observe ovarian structures with either a transabdominal or transrectal approach. Trans-abdominal imaging is generally done with a 5.0 MHz probe which sacrifices a degree of image quality for depth of penetration and can be hindered by sow movements. Trans-rectal imaging generally provides the best picture quality, and size restrictions due to the pelvic area of gilts and some sows can be overcome by utilizing a stabilizing rod, which allows “hands-free” probe insertion (Knox and Althouse 1999). Unlike some other domestic species, follicle size during estrus does not appear to be a reliable predictor of ovulation time in swine (Soede and Kemp 1999). The ovulation status can be confirmed by the presence of follicles (6.5 mm to 7.5 mm) versus corpora hemorrhagica or corpora lutea on the ovaries (Knox and Althouse, 1999). With appropriate training and technique, transrectal RTU makes it possible to confirm pregnancy as early as 16 to 18 days after breeding, allowing the producer to further reduce non-productive sow days by identifying open sows for rebreeding during their normal return to estrus (Knox and Althouse 1999).

Certain pathologies such as ovarian cysts and pyometra can be identified with RTU. Even though the majority of ovarian cysts regress spontaneously, they are still likely to be a source of subfertility (Waberski 2000). Definitive diagnoses made with RTU facilitates the producer’s ability to make management decisions based on accurate information on previously un-observable antemortem criteria. Recently, the ability of RTU to visualize the accessory sex glands of boars as a diagnostic aid in assessment of these was demonstrated (Clark and Althouse 2002). Transvaginal RTU guided oocyte aspiration has also been successfully demonstrated (Caamano et al. 2002).

IX. Reproductive Pharmacology

The Animal Medicinal Drug Use Clarification Act (AMDUCA) governs the use of reproductive hormones in swine as in other species. In the USA, extra label drug use (ELDU) is not permitted for the expressed purpose of improving reproductive efficiency, but only for therapeutic purposes (Kuster 2001). For this reason, only those drugs approved by the FDA for use in swine will be discussed in this section.

Gonadotropins: The combination of 400 IU eCG and 200 IU hCG (PG 600®, Intervet, Millsboro, DE) has a label claim for use in the swine breeding herd to induce estrus in pre-pubertal gilts. The usual response observed after PG600® injection is 70% of gilts exhibiting estrus by 7 days, and 70% of these having a regular estrous cycle (Kirkwood 1999). A second label claim for PG 600® is the induction of estrus in sows when given at the time of weaning.

Additionally, some producers treat sows that do not display estrus by 7-10 days after weaning. Groups of sows treated with PG 600® at the time of weaning will generally respond with a shorter and more synchronous onset of post-weaning estrus, although this effect is more noticeable if a specific problem is being targeted such as primiparous sows or the summer season (Kirkwood 1999). The response of sows treated with PG 600® at 7 to 10 days post-weaning is much more variable, and depends on the hormonal status of the individual animal. A 70% estrus response is seen if the sow is truly anestrous. If the sow has already ovulated and the estrus was unobserved, corpora luteal progesterone production will block the behavioral response (Kirkwood 2001). Utilizing a progesterone ELISA or RTU in conjunction with PG 600® would help diagnose the problem of sows with extended wean-to-estrus intervals.

The injection of GnRH into sows at the time of breeding or at day 12 after breeding has been shown to influence their pregnancy rate, but only under conditions of otherwise poor fertility (Peters et al. 1997; 2000). The mechanism involved was not determined but it may involve the myometrial production of PGE_2 (Ziecik et al. 2000). If repeatable, this therapy may have an application in the alleviation of seasonal infertility where a presumptive inadequate luteal support leads to an increase in irregular returns.

Prostaglandin: Prostaglandin F2α (PGF) is commonly used in swine production for the induction of farrowing. Lutalyse® (Pharmacia, Kalamazoo, MI) is approved for use IM at the level of 10 mg (2ml) up to 72 hours prior to the expected farrowing date (Bradford 2000). The goal of induced farrowing is to have trained personnel available to assist during farrowing. A number of strategies have been employed to time the onset of farrowing with greater precision, such as the injection of 10 IU of oxytocin 24 hours after Lutalyse® is administered (Bradford 2000). However, the use of oxytocin can result in obstetric problems (Kirkwood 1999). Recently, the efficacy of two injections of PGF ("split-dose") has been demonstrated (Kirkwood 1999). PGF can also be used for estrus synchronization by aborting bred females with 10 mg of Lutalyse® at 14 to 35 days of gestation. Like farrowing induction, an improved response is obtained by using a split dose of 10 mg, 12 hours apart to induce abortion and return to a fertile estrus (Almond 2001).

Oxytocin: In addition to its involvement in farrowing induction programs, oxytocin is used to induce uterine contractions in cases of uncomplicated uterine inertia. Doses should be relatively low (5 to 10 IU) in order to avoid strong asynchronous uterine contractions, which can be both painful and unproductive (Almond 2001). Oxytocin can also be administered to initiate milk let-down in the treatment of lactational insufficiency, although success rates are low (Smith 1985).

Corticosteroids: The inclusion of corticosteroids in the farrowing induction protocol has been shown to accelerate piglet delivery (Bilkei 1992) and reduce neonatal mortality. Additionally, injecting the periparturient sow with corticosteroid may increase piglet viability and subsequent neonatal growth rate (De Rensis et al. 2002).

Progestogens: Currently, no progestogens are approved for use in swine in the USA. If available, an orally active progesterone product would be extremely useful in gilt estrus synchronization programs, and delaying and synchronizing the post weaning estrus of sows (Shipley 2001).

X. Disease

Swine are susceptible to many diseases that adversely affect reproduction. For this discussion the focus will be on major diseases of contemporary importance including porcine reproductive and respiratory syndrome, swine influenza and porcine circovirus.

Porcine reproductive and respiratory syndrome (PRRS): Porcine reproductive and respiratory syndrome has been the most infamous swine disease of the past decade (for review, see Benfield et al. 1999; Dee et al. 1999; Mengeling et al. 2000). PRRSV is an Arterivirus, and is classified in the same genus as equine viral arteritis virus (Benfield et al. 1999). The first clinical case of PRRS was identified in North Carolina in 1987, and since that time has spread throughout North America (Mengeling et al. 2000). The clinical signs of PRRS can vary wildly depending on such factors as virulence of the infecting strain(s), immune status of the herd, and management factors. Most PRRSV-induced epidemics of reproductive failure in naïve herds include late-term abortions and the delivery of early, full-term or late gestation litters containing some or all of the following: mummified fetuses, late-term dead fetuses, weak, unthrifty pigs and normal pigs (Benfield et al. 1999). In the fall of 1996 the emergence of 'atypical' PRRS resulted in severe epidemics distinguished by abortions at all stages of gestation, fever, inappetence, recumbency, and even sow mortality in addition to the typical clinical signs (Mengeling et al, 2000). Since that time, the prevalence of 'atypical' PRRS seems to have dissipated as quickly as it arose. Porcine reproductive and respiratory syndrome virus can be transmitted horizontally and vertically, and there is field evidence that implicates area spread via aerosol transmission, although this last route has not been confirmed experimentally (Mengeling et al. 2000). Vaccination can be effective at minimizing reproductive failure due to PRRSV, with the type of vaccine used (MLV versus inactivated) and the antigenic similarity to the field strain of exposure being important factors impacting the success of this preventive measure. Diagnostic tests commonly employed in PRRS investigation and monitoring include PCR, virus isolation, and ELISA; each of which supplies different information and, thus, is applied in specific circumstances.

Biosecurity procedures have long been a part of swine health management programs, and have evolved over the course of time from focusing on the prevention of relatively well-understood diseases such as pseudorabies, swine influenza and transmissible gastroenteritis to the control of PRRSV. As such, isolation periods for replacement breeding stock have steadily increased from an historical standard of four weeks to more aggressive periods of 45 to 60 days or longer as new knowledge has been gained as to the duration of viral shedding of PRRSV. Since PRRSV can be intermittently shed by boars and transmitted via extended semen (with virus detected in boar semen by PCR as much as 92 days post-infection after experimental challenge) it is difficult to justify the use of semen from previously PRRSV-positive boars in naive sow herds (Christopher-Hennings, 2001). The current emphasis on PRRSV control is in the area of eradication, with various programs being devised to achieve this elusive goal including test-and-removal, partial

or total depopulation, and changes in pig-flow and gilt introduction practices (Dee et al. 2000; Hassing et al. 2000; Torremorell and Baker 2000).

Swine influenza virus (SIV): Swine influenza virus is not new, it being first described in 1918 (Easterday and Van Reeth 1999). Swine influenza is caused by type A influenza viruses in the family Orthomyxoviridae and sub-types are designated by two distinct kinds of major surface antigens: hemagglutinin (H) and neuraminidase (N). The H1N1 serotype has been the predominant subtype in North America until very recently. In 1994, an H3N2 serotype was identified in Canada (Bikour et al. 1994). The first clinical case of H3N2 in the USA was detected from an abortion storm in August of 1998 (Erickson 2000), followed by sweeping SIV epidemics across the major pig rearing areas of the country later that fall. Since the surface proteins of H1N1 and H3N2 are dissimilar, vaccinating with H1N1 vaccine provided little or no protection to the new virus. The new strain of SIV appears to be more virulent than H1N1, inducing severe clinical signs, even in adult swine, which include abortions and sow mortality (Wagner 1999). Commercially available bivalent swine influenza vaccines have recently been developed as an aid in the prevention of SIV caused by both H1N1 and H3N2 serotypes (e.g. Schlueter et al. 2001).

Porcine circovirus 2 (PCV2): Porcine circovirus 2 is a recently described agent associated with postweaning multisystemic wasting syndrome (Ellis et al. 1998). More recently, this virus has been isolated from aborted fetuses from a start-up farm experiencing late term abortions and stillbirths (West et al. 1999). One of the fetuses had severe dilated myocarditis, presumably the proximate cause of death. Tests for porcine parvovirus, PRRSV, encephalomyocarditis virus, and enterovirus were negative. The ability of PCV2 to replicate and cause pathologic lesions in fetuses when infected at different times during gestation has been confirmed (Sanchez et al. 2001). The true extent of the involvement of PCV2 in herd fertility problems remains unknown.

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