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NEW TOOLS FOR MANAGING REPRODUCTION

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SUMMARY

A series of biological events must occur before pregnancy can be established. Female cattle are anoestrus after parturition and cannot become pregnant. Once animals begin to cycle, then the oestrous cycle can be effectively controlled through hormonal treatments. Multiple drugs can be delivered into the vagina by plastic devices that contain physical pumps. These new devices will decrease animal handling before insemination. New chemical formulations may make synchronisation products easier to use as well. Classical technologies for oestrous detection depend on mounting activity of cows. Newer methods of electronic oestrous detection measure the number of mounts that each cow receives. More modern approaches to oestrous detection capitalise on animal biology instead of the behavioural patterns of the cow.

Artificial insemination is widely practiced in animal agriculture. Increasing sperm lifespan in the female reproductive tract would greatly simplify artificial insemination, but a method to increase sperm lifespan has not been developed. *In vitro* embryo production has the capacity to provide a large number of embryos at a relatively low price. These low-price embryos can be used on low fertility cows that are incapable of producing a viable embryo.

Greater intensification is associated with earlier pregnancy diagnosis. Ultrasonography can be used to detect earlier pregnancies in cattle. A blood test for pregnancy may allow for even earlier diagnosis. If pregnancy tests for maternal recognition proteins can be developed, then non-pregnant cows could be identified 18 days after first insemination. Beginning a rapid resynchronisation program on day 18 would lead to a second insemination of non-pregnant cows within 21 days of first service.

The advent of genome technologies has placed renewed emphasis on understanding the relationship between animal genetics and reproduction. Genetic selection for improved fertility is possible. New genes that have major effects on reproduction have been discovered.

A combined approach involving reproductive genetics, oestrous cycle control, *in vitro* embryo production, early pregnancy detection and automated animal handling will improve reproductive management in future herds.

Keywords: oestrus, insemination, pregnancy, cattle

INRODUCTION

Reproduction is an important part of farm animal operations. For dairy cattle, pregnancy leads to the birth of a calf that renews the lactation cycle. For beef, sheep and pig producers, pregnancy leads to the production of young stock, the sale of which is a primary income for the producer. Farmers achieve greater reproductive efficiency through a variety of methods. One way to increase reproductive efficiency is to increase herd pregnancy rates (i.e. the percentage of pregnant animals in the herd after a specified breeding period). A second way to increase reproductive efficiency is to increase the number of offspring born per mother (primarily applicable to litter-bearing species; sheep and swine). Genetic selection can improve reproductive efficiency. For example, maternal lines in swine are selected for greater litter size. Mating schemes in sheep capitalise on genetic mutations that increase follicular development and litter size. The selection pressures for farm animals are not always applied to reproductive traits and, in some cases, alternative selection priorities can antagonise reproductive events. This is particularly true for dairy cattle (Lucy 2001; Diskin *et al.* 2003). The rapid progress in genetics and management in the dairy industry has led to cows that produce more milk. Sustained milk production (multiple lactations) of individual cows depends on their ability to become pregnant. Unfortunately, the genetic trend for greater milk production is unfavorable toward reproduction. Thus, farmers must do more to achieve pregnancy in high-producing dairy cattle.

A series of biological events must occur before pregnancy can be established. Fertile ovarian cycles must be established so that viable ova are produced. Ovulation is preceded by overt signs of oestrus. If artificial insemination (a tool for rapid genetic progress) is employed, then signs of oestrus must be seen by the farmer so that the animal is inseminated around the time of ovulation. Inseminated animals need to be checked for pregnancy so that non-pregnant animals can be identified and re-enter the breeding program. This paper will review some of the latest technological developments that can be used to manage reproduction in farm animals. Practical application of the methods will also be discussed. For simplicity, methods applied to beef and dairy cattle will be presented. Sheep and swine species will be referenced where appropriate.

CONTROL OF THE OESTROUS CYCLE

Oestrous synchronisation methods

Female cattle have an infertile period (anoestrus) after parturition during which they cannot become pregnant because they do not ovulate (Rhodes *et al.* 2003). This period can last for 30-40 days in dairy cattle, and may be longer in beef cattle (suckling-induced anoestrus). Nutrition plays a major role in determining the length of anoestrus in both beef and dairy cattle (Lucy 2003). The ability to control the length of anoestrus by pharmacological intervention is well-developed in cattle. Cows can be treated with progesterone to induce fertile ovulation. Once animals begin to cycle, then the oestrous cycle can be controlled through additional hormonal interventions (Thatcher *et al.* 2001; Diskin *et al.* 2002; Rhodes *et al.* 2003). These include prostaglandin $F_{2\alpha}$ (PGF), used to regress the corpus luteum; oestradiol, used to induce oestrus; and gonadotropin-releasing hormone (GnRH) used to cause ovulation. Progesterone can also be applied to block ovulation until the desired time. A variety of protocols (oestrous synchronisation programs) have been developed for inducing fertile oestrus.

Most synchronisation protocols involve a series of 3-5 intramuscular injections spaced at specific intervals (2-7 days). The protocol may also involve the insertion and removal of a progesterone-releasing device. In some systems, the herd can be handled collectively. In other words, all of the cows are given injections on the same day (whole-herd synchrony programs). This facilitates animal management because every animal receives the same injection. Whole-herd synchrony programs work well for seasonal calving herds where a compact calving pattern for the entire herd is desired. Industry-wide application of whole herd synchrony has been slow, however, because of their perceived expense and slightly lower conception rates when applied to low-input systems. In continuous calving herds (commonly found in North America and becoming more common in Australia) individual cows must be treated because only a small percentage of the herd calves on any given day. The treatment of individual cows greatly complicates the synchronisation program, particularly when several injections are required. The complexity can be reduced somewhat by grouping cows in weekly cohorts for injections.

Single devices for multiple hormone application

Progesterone, oestradiol, and PGF can be delivered into the vagina by plastic devices that contain a physical pump. The pump accurately controls the time of administration. Packaging all of the required treatments into a single intravaginal insert greatly simplifies the process because cows are only handled twice; once to insert the device and once to remove the device and inseminate. Two systems have been designed and tested for this purpose (Rathbone *et al.* 2001). Early prototypes were electronic devices with a physical pump delivering a variety of hormones. More advanced prototypes use hydrolytic gas for the same purpose. Both systems are capable of delivering pharmaceuticals intravaginally at predetermined intervals. Continued development should lead to commercialisation of the devices.

Modification of existing compounds and existing delivery devices

The 4 primary drugs used to synchronise oestrus are PGF, GnRH, oestradiol, and progesterone. Each can be chemically modified to change half-life, potency, and preferred route of administration. Progestogens can be injected intramuscularly, placed into the vagina, placed subcutaneously (norgestomet capsule) or fed (melengestrol acetate). Oestradiol can be administered as oestradiol valerate, oestradiol cypionate (used in the USA) or oestradiol benzoate (used in New Zealand and Australia). Gonadotropin-releasing hormone causes ovulation when given as a single dose, but potent GnRH agonists given over extended periods cause infertility by inhibiting luteinising hormone (LH) secretion (D'Occhio *et al.* 2000). Absence of reproductive cycles is desired for certain production systems. In feedlot heifers, for example, oestrus decreases growth performance. Sustained release formulations of GnRH can be administered to feedlot heifers to decrease the incidence of oestrus and improve growth (D'Occhio *et al.* 2002). Likewise, early ovulation in postpartum cows may lead to poor uterine health and subsequent infertility. Long-acting GnRH agonists could be used to suppress ovulation in postpartum cows and improve reproductive outcomes during the breeding period.

Intravaginal devices containing progesterone consist of silicone rubber applied to a nylon backbone. The progesterone is released over time from the silicon. Although these devices are robust, the delivery kinetics are not optimal. A large bolus of progesterone is released initially, and then there is a slow decay of progesterone release. Used progesterone devices contain a large amount of residual progesterone. The CIDR device (InterAg, Hamilton, NZ) was redesigned for the US market so that less progesterone was used in each device (Rathbone *et al.* 2002). The redesign decreased the up-front progesterone load, but also decreased the residual progesterone left in used devices. Progesterone pumps (described above) have the advantage of a more precise payout of progesterone. The development of biodegradable polymers for the purpose of progesterone delivery is another conceptual advance (Rathbone *et al.* 2001). The safe disposal of used progestogen-releasing devices is easier because the polymer is biodegradable.

MONITORING THE OESTROUS CYCLE AND OVERT SIGNS OF OESTRUS

Farmers perform artificial insemination within 8-12 h of detected oestrus. Many beef and dairy operations do not use any form of oestrus synchronisation. The opportunity for insemination depends on the reproductive cycle and observed signs of oestrus. Cows treated with oestrous synchronisation programs need to be inseminated after treatment as well. The insemination can either be done at a fixed time after the last injection (timed AI) or can be done after the cow is observed in oestrus. Fixed-time AI programs are popular in North America because oestrous detection is not required. Although timed AI programs have distinct advantages, conception rates are low relative to insemination at observed oestrus (Jobst *et al.* 2000). Regardless of the system used (e.g. no intervention, hormonal intervention), the highest pregnancy rates are achieved in cows showing signs of oestrus. Higher rates of oestrous detection, therefore, improve reproductive efficiency in beef and dairy herds.

Monitoring the overt signs of oestrus

Cows in oestrus have a characteristic behaviour in which they will stand still when mounted by a herd mate. A farmer will schedule an insemination when he observes this behaviour. The frequency of the behaviour largely depends on the floor surface (Vailes and Britt 1990). Confinement-housed cows that live on concrete are less likely to display oestrus compared with cows living on pasture. The belief is that slippery surfaces like concrete create a fear of falling in cows that overrides their desire to mount other cows. It is also possible that feet and leg problems (characteristic of confinement-housed cows) impede mounting behaviour. Cows in oestrus also have the capacity to attract cows to them and stimulate mounting behaviour. The physical attraction is mediated by pheromones emitted from the vagina. New and more sensitive methods to detect oestrus could have important implications for reproduction, particularly in systems where cows are housed indoors on concrete.

Mounting causes pressure and physical abrasion of the tail head. Classical technologies for oestrus detection capitalise on this fact. Tail heads can be painted, and the loss of paint (indicative of mounting activity) can be evaluated daily (paint score system; 5 = all paint to 0 = no paint). Cows without paint are inseminated. The problem with paint scoring is that it is a subjective system. A cow with a 5 is definitely not in oestrus, and a cow with a 0 is in oestrus, but what about the cow with a paint score of 2? Patches with built-in pressure activated pouches (either red in color or florescent light-emitting) offer some advantages because their evaluation is less subjective, but they are more expensive than the simpler tail paint system. Either system requires visual inspection by a human being at least once daily, an obvious disadvantage, particularly in large continuous-calving herds.

The HeatWatch system (Nebel *et al.* 2000) was designed to automate the process of oestrous detection. A transponder is mounted within a nylon patch glued to the tail head of the cow. Pressure from mounting depresses a button on top of the transponder. The transponder sends a signal to an antenna mounted in the barn, which is linked to an on-farm computer. The number, duration, and time of day for each mount are displayed for every cow in the herd. An algorithm is used to predict cows in oestrus (minimum number of mounts over a predetermined period). The HeatWatch system provides accurate data on the expression of oestrus for individual cows. Its commercial application has been slowed by the up-front cost of setting up the system, and also by the day to day monitoring of patches on individual cows. The technology works well, but must be managed to extract the greatest value from the system.

Cows in oestrus have increased activity. The level of activity of an individual cow can be measured by using pedometers (monitoring distance walked) or neck chain monitors (measuring movement of the head). The level of activity is indicative of oestrus (Redden *et al.* 1993). Activity data are read by an antenna when cows enter the milking parlor. Cows with high activity relative to their previous baseline may be in oestrus. Although pedometers or neck chain monitors are less accurate for predicting oestrus than HeatWatch, they may be more robust and simpler to use. The up-front cost of setting up the system and lower accuracy of oestrus detection are the primary detractions for activity monitoring.

Biological monitoring of the oestrous cycle

HeatWatch and activity systems have achieved commercial application, but both are based on overt signs of oestrus. Cows that have short periods of oestrus or receive very few mounts are difficult to detect. More modern approaches go beyond oestrus behaviour, and capitalise on animal biology and physiology that does not depend on the behavioural patterns of the cow. These biological approaches have clear advantages for modern dairy production systems where the intensity of oestrus may be less. Intravaginal temperature monitoring is an example of a physiological measurement that can be used to detect oestrus (Kyle *et al.* 1998). Radiothermometers are typically placed in the vagina, but could theoretically be placed within the rumen or abdomen of the cow. Body temperature is measured when cows enter the milking parlor. Cows in oestrus have an increase in body temperature. Body temperature monitoring is feasible because it can be automated for hands-off monitoring of cattle. This is a critical feature of any successful reproductive monitoring system. Sick cows have an increase in body temperature, so body temperature monitoring has added value as a tool for assessing the health of individual cows.

Milk progesterone concentrations can be used to predict the time of oestrus, and also identify pregnant and non-pregnant cows. Either radioimmunoassay or ELISA can be used to measure milk progesterone, but neither method can achieve the high-throughput needed for daily monitoring of individual cows. Recent advances in biosensor technology may lead to the development of progesterone sensing devices capable of measuring milk progesterone in real-time (Velasco-Garcia and Mottram 2001). Individual cows can be monitored for cyclicity by placing the sensor in the milk line. The time of oestrus can be predicted from the decrease in progesterone after luteolysis. Although the system has not evolved to commercial application, the biosensor concept makes sense given the need for automated systems for oestrous detection. A patch that measures sweat ion concentrations that are correlated with changes in reproductive hormones around oestrus was recently tested in Canada (Pheromone Science Corporation, Toronto). Like milk progesterone, the patch device may have the greatest sensitivity for oestrous detection in cattle because it indirectly measures reproductive hormone concentrations.

Other considerations

Vaginal electrical resistance can be used to detect cows in oestrus (Foote *et al.* 1979). A probe is inserted into the vagina of each cow and electrical resistance is measured. Although the system can detect oestrus, the time and effort required for daily testing of individual cows eliminates any chance of large-scale commercial application. Many of the cows detected in oestrus by electrical resistance will have already shown overt signs of oestrus. Thus, a large number of cows are tested to detect the relatively small percentage of cows that show no sign of oestrus whatsoever. The hassle factor was the greatest limitation to the wide-scale implementation of the method. Likewise, an electronic nose that could measure perineal odors was tested and shown to be somewhat successful in the late 1990's (Lane and Wathes 1998). An optimised detection method still needs to be developed. But perhaps the more critical need is a mechanical method to sample perineal fluid daily from individual cows. It is unlikely that any system of automated oestrous detection will be commercialised successfully unless it is accurate, durable, inexpensive, and easy to use (i.e. minimal hands-on management).

Most automated systems of oestrus detection require computerised integration of information from a variety of sources. The computer algorithms used to predict oestrus may ultimately determine the accuracy of the system. Low false positive and false negative rates are needed. Some algorithms are too liberal (too many false positives) and cows that are not in oestrus are inseminated. Others are too stringent (high false negative rates) and oestrual cows are missed. Fuzzy logic may help in this regard. A recent study demonstrated the incidence of false positive alerts for a pedometer system could be reduced considerably by the use of fuzzy logic (de Mol and Woldt 2001).

ARTIFICIAL INSEMINATION AND EMBRYO TRANSFER

Artificial insemination is widely practiced in animal agriculture. Semen may be fresh (i.e. diluted after collection and used) or frozen (i.e. frozen shortly after collection and thawed immediately before use). Systems for the collection, dilution, and distribution of fresh semen are practiced in New Zealand. Fresh semen can be marketed at a higher dilution and, therefore, increases the number of inseminations from a single ejaculate. Frozen semen can be stored indefinitely, but dilution rates are less than those used in fresh semen. The capacity to collect semen from a small number of sires for use on a large number of damns has had major implications for the worldwide dairy industry. North American dairy cows are highly inbred with an effective population size of fewer than 30 individuals (Weigel and Lin 2002). The use of North American genetics in New Zealand and Australia has created concern among dairy farmers who must attempt to manage larger cows in lower body condition. Artificial insemination in swine is routinely practiced, but a reliable method to freeze swine semen has not been discovered. Although fresh swine semen from superior sires is marketed, the relatively short shelf-life makes the system cumbersome and expensive. Swine producers do, however, collect and dilute semen from their own boars.

Semen placement in the reproductive tract

Techniques used for artificial insemination have not changed a lot since the original development of the method. Semen is deposited in the uterine body of cattle. In swine, semen is traditionally deposited into the cervix. Semen placement is a contentious topic in cattle because some argue that depositing semen deep within the reproductive tract (horn breeding) will increase conception rate (Hunter 2003). The efficacy of horn breeding may ultimately depend on the experience of the inseminator. Inexperienced inseminators that unknowingly place semen into the cervix may benefit from the practice of horn breeding. Until recently, passing an insemination pipette through the swine cervix was considered unfeasible. Better catheter design, however, has enabled the placement of semen directly into the uterus of swine. An even longer catheter can be used to place the semen near the oviduct (Martinez *et al.* 2004). Uterine insemination reduces the sperm dose by approximately one-third. Placing the semen near the oviduct reduces sperm-dose further. Superior boars, therefore, can serve a greater number of sows.

Increasing sperm life

Farm animals are inseminated around the time of oestrus (see above). The timing of insemination is critical because sperm cells must capacitate before fertilisation, and they also have a finite lifespan in the reproductive tract. Ovulation in cattle is predictable because cattle ovulate about 28 h after the onset of oestrus. A single insemination can be used if the onset of oestrus is known. The time of ovulation relative to the onset of oestrus is more variable in swine. Thus, 2 inseminations are required. Reproductive management would be simpler if the time of ovulation could be accurately predicted (particularly in swine), or if sperm had a longer lifespan in the reproductive tract. The timing of ovulation can be improved somewhat by administering GnRH at insemination. The GnRH treatment increases conception rate in repeat-breeder cattle that may have delayed ovulation relative to oestrus (Stevenson *et al.* 1990). The possibility of increasing sperm lifespan is conceptually feasible because sperm can survive for long periods within the female reproductive tract of some species. For example, sperm survive within female chickens for over a week. If cattle were similar to chickens, then a single weekly insemination would suffice. Even a small increase in sperm survival time could have major implications because the time of insemination could be less-precisely timed with oestrus. A variety of possibilities have been investigated (Nebel *et al.* 1993), but at this time a method to increase sperm life in the reproductive tract has not been developed.

Separation of X- and Y-chromosome bearing sperm
Selecting the gender of offspring before mating would greatly increase the efficiency of production systems. In dairy, for example, the number of potential replacement heifers could be increased by inseminating with X-bearing sperm. Bull mothers could be inseminated with Y-bearing sperm to guarantee male offspring. The physical selection of X- and Y-bearing sperm is possible and is practiced commercially (Seidel 2003). Standard florescent cell sorting technology is used. Sperm cells are stained with a DNA dye, and the relative DNA content of X- and Y-bearing sperm is used as a basis for selection. The efficiency of selection varies with species and depends on the inherent difference in DNA content of the X- and Y chromosome. Separation of X- and Y-bearing sperm is possible for farm animals, and a highly skewed gender ratio can be achieved. The efficiency of the process, however, is low. Florescence-based cell sorting is too slow to handle the large number of cells produced from a single ejaculate. Only a handful of straws can be produced at 1 time. Deep uterine horn insemination can reduce the required number of sperm cells per insemination, but production capacity is still too low for general application of the technology. The efficiency of the process appears to be at a maximum for individual cell sorters so the only way to increase production is through parallel production (multiple sorters working at the same time). Although separation methods may not be ready for general application, they are probably sufficient to meet the needs of some producers.

Increasing fertility with embryo transfer
Perhaps the greatest recent advances in reproductive biology have occurred in the area of embryo development and cloning. It is now possible to clone a variety of animals from somatic cells. Farm animals (sheep, cattle and swine) contributed to some of the earliest successes in this area. Cloning is a tool for advancing genetic progress on the maternal side, and is commercially applied to elite cattle.

Some causes of infertility in cattle are manifested at the ovarian level prior to ovulation or during the earliest stages of embryonic development. For example, heat-stressed cattle have low conception rate after artificial insemination. The poor conception rate can be explained by poor oocyte quality and poor embryonic development in heat-stressed cattle. Embryo transfer in heat-stressed cattle improves conception rate relative to artificial insemination (Hansen *et al.* 2001). There is a need, therefore, for low cost bovine embryos that can be transferred into cows with inherent low fertility.

Superovulation has been practiced for several decades, and research has focused on methods to increase embryo production from individual cows (Hasler 2003). Unfortunately, the rate of embryo production from superovulation has not changed since its original development. The failure to increase embryo production probably arises from the fact that methods to increase the recruited pool of follicles have not evolved. *In vitro*-produced (IVP) embryos have the capacity to meet the demands for large scale embryo production (Galli *et al.* 2003). Oocytes are collected from slaughterhouse ovaries, matured and fertilised *in vitro*, cultured, and then packaged for fresh or frozen transfer. It is theoretically possible to perform a DNA test, and market gender-selected IVP embryos.

In vitro embryo production has the capacity to provide a large number of embryos at a relatively low price. Problems with low conception rates after embryo transfer and abnormal foetal development (e.g. large calf syndrome) remain to be solved. Semen from superior sires is used, but the maternal genetics are generally unknown because the ovaries are collected from the slaughterhouse. Improving the efficiency of IVP, increasing conception rate after embryo transfer, and guaranteeing embryo genetics (breed) would probably increase the commercial use of IVP embryos for low-fertility cattle.

Methods to increase fertility after first insemination
A number of different approaches have been devised to increase pregnancy rate after first insemination (Binelli *et al.* 2001). Pregnant cows have higher progesterone concentrations than non-pregnant cows. Treating cows with GnRH or human chorionic gonadotropin (hCG) after insemination increases luteal mass and blood progesterone concentrations. The effect of GnRH or hCG treatment on conception rate, however, has been equivocal. Feeding dairy cows polyunsaturated fatty acids may block uterine prostaglandin synthesis and antagonise the luteolytic process. Blocking luteolysis may increase embryo survival in at-risk cows. Fats are typically fed at a low-level in US dairy diets. Polyunsaturated fatty acids could be substituted for saturated fatty acids within these systems. The polyunsaturated fatty acids have similar energy and also the added reproductive benefit.

PREGNANCY DETECTION

People erroneously think that the value of a pregnancy exam is in detecting pregnant animals. The true value is in detecting non-pregnant cows so that they can re-enter the breeding program. The simplest form of pregnancy detection is to wait a full gestation length. Pregnant animals eventually give birth. Non-pregnant animals do not. Although the inherent inefficiencies are obvious, the aforementioned method may be the most common in animal production. More intensive beef producers will do pregnancy diagnosis in the autumn at weaning. Non-pregnant cows and weaned calves can be sold at the same time. Greater intensification is associated with earlier pregnancy diagnosis. For example, dairy producers perform pregnancy detection at the end of the breeding period or at a predetermined interval after insemination. The greatest efficiency is achieved when pregnancy detection is done during the breeding period, when non-pregnant cows can be identified and re-inseminated. Farmers routinely do this when they check for returns to oestrus in inseminated animals. Finding the cows that return to oestrus is problematic for modern dairy producers because high rates of early embryonic death have lead to a phantom cow phenomenon (Cavalieri *et al.* 2003). Phantom cows are not pregnant and do not a return to service. Their pregnancy failure is detected at pregnancy exam when the cow may no longer be eligible for breeding. In-line progesterone sensing (mentioned above) would help farmers because progesterone concentrations could be used to identify pregnant and non-pregnant cows.

Ultrasound pregnancy diagnosis

Pregnancy exams in cattle are done by feeling for the foetus within the uterus. Thirty-five to forty day pregnancies can be detected by this method. Ultrasonography can be used to detect earlier pregnancies in cattle (25-28 days in a field situation) (Fricke 2002). Although slow to develop, ultrasound examination is becoming more common for cattle. In New Zealand, ultrasound is practiced on large herds for the purpose of accurately staging pregnancy in individual cows. Ultrasound examination for pregnancy diagnosis is already a standard practice in the swine industry. Ultrasound has distinct advantages relative to rectal palpation because embryos are detected earlier and the risk of accidental abortion is less. Non-viable foetuses that do not have a heartbeat can be identified and cows rechecked to confirm subsequent foetal loss.

Blood tests for bovine pregnancy

Ultrasound pregnancy examination involves the physical handling of the cow. Although early pregnancies (less than 28 days) can be detected the time required for the ultrasound exam may be too long when a large number of cows are scheduled for exam. Time is not a factor for ultrasound detection of later pregnancies because the embryo is larger. A blood test for pregnancy could simplify detection, and also allow for earlier diagnosis. The bovine placenta produces pregnancy-associated glycoproteins (PAGs) and PAGs can be detected in the blood by about 25 days after breeding (Perenyi *et al.* 2002). Tests for PAGS have been commercialised, but the uptake has been slow, perhaps because the advantages of an early test are not clear. Simple methods to re-inseminate non-pregnant cows are needed.

We are experimenting with a method of rapid resynchronisation that would allow the re-insemination of non-pregnant cows within 2 days after a day 28 pregnancy test. The test could be a PAG blood test or an ultrasound examination. Using rapid resynchronisation on non-pregnant cows ensures that non-pregnant cows are re-inseminated shortly after pregnancy diagnosis. Most non-pregnant cows are in oestrus 19-23 days after first insemination. If pregnancy diagnosis is done at approximately day 28, then non-pregnant cows will be on days 5-9 of the subsequent oestrous cycle, a period when a PGF-responsive corpus luteum and a dominant follicle are present on the ovary. Injecting PGF and GnRH in a 2-day sequence led to timed AI pregnancy rates that were similar to first insemination (Meyer and Lucy, unpublished results; Stevenson *et al.* 2003). Thus, the first oestrus synchronised ovarian follicles and corpora lutea during the second oestrous cycle. The corpus luteum could be regressed and the first wave dominant follicle ovulated in a rapid sequence suitable for timed insemination.

Detecting pregnancy before the natural return to oestrus

Early pregnancy diagnosis will improve reproductive efficiency if non-pregnant cows are re-inseminated shortly after diagnosis. Considerable excitement was generated by the marketing of the Early Conception Factor (ECF) test for dairy cattle. The ECF test was supposed to detect pregnancy within 2 days after fertilisation and would theoretically identify failed inseminations. Independent evaluation soon demonstrated that the test did not work (Cordoba *et al.* 2001), and a major breakthrough in pregnancy diagnosis was not realised. Diagnosing pregnancy at around the time of maternal recognition of pregnancy (days 15-20) may be a more viable approach. A variety of uterine genes are up-regulated at that time (Hicks *et al.* 2003; Austin *et al.* 2004). If early pregnancy tests can be developed for these proteins, then pregnant and non-pregnant cows could be identified 18 days after insemination. Rapid resynchronisation (PGF-GnRH) could be practiced because a corpus luteum and second-wave dominant follicle are on the ovary. Pregnancy testing cows on day 18 and applying rapid resynchronisation would enable the re-insemination of non-pregnant cows 21 days after first service. Thus, the re-insemination interval is equal to the normal return to oestrus. Combining a timed AI at first insemination with rapid resynchronisation would limit reproductive management to 3 days a week without the need for oestrous detection. Every non-pregnant cow would be inseminated once every 21 days.

IMPROVED GENETICS FOR REPRODUCTION

Selecting for better fertility

Reproductive traits have low heritability, but the coefficient of variation of reproductive traits is very large (Royal *et al.* 2002). Therefore, genetic selection for improved fertility is possible. The recent reproductive decline in dairy cattle has led to the incorporation of fertility traits into breeding values for dairy bulls. There are negative genetic correlations between daughter fertility and milk yield. Although progress toward greater milk production may be less, appropriate weighting for fertility in selection indices should lead to greater profitability when better fertility bulls are used. The heritability of body condition in lactating cows is considerably higher than the heritability for reproductive traits. Cows in good body condition have better reproduction. The use of body condition in selection indices may be a faster way to improve reproduction in dairy cows. The appearance of better-conditioned cows may also be viewed more favorably by the general public.

New genes for reproductive traits

The advent of genome technologies (e.g. large scale DNA sequencing, microarrays, bioinformatics) has placed renewed emphasis on understanding the relationship between animal genetics and reproduction. Prolificacy traits in sheep have now been traced to specific mutations that affect follicular development and litter size (Galloway *et al.* 2002). Several different prolific lines of sheep have mutations within the same gene. Traditional selection processes, therefore, converged upon a single gene that affected follicular growth. A mutation in the oestrogen receptor (ESR gene) is associated with greater litter size in pigs (Rothschild *et al.* 1997). The large effect of the gene (nearly 1 pig per litter) is remarkable given the quantitative nature of reproductive traits. Variants of a heparin-binding protein found in seminal plasma (fertility associated antigen; FAA) are correlated with greater fertility in bulls (McCauley *et al.* 1999). Lateral flow tests are now available to test individual bulls on-farm (Reprotec; www.reprotec.us). Before the discovery of FAA, low fertility bulls with seemingly normal semen would be unknowingly used in breeding programs. Bulls can now be tested for FAA, and those with the wrong antigen (low fertility) can be culled.

The key to any new genetic discovery is to develop a method to test animals, and then use the information in breeding schemes. Genetic tests for each of the above fertility genes have been developed and they can be used to improve fertility. Prolificacy genes in sheep achieve the desired phenotype in heterozygous animals. Animals carrying both mutations are infertile. Newborn females can be tested for genotype to determine which animals should eventually be used in the breeding herd. Widespread use of some markers quickly leads to fixation of the gene in the target population. Once the gene is fixed (i.e. all individuals carry the desired trait) then the marker has no value in the breeding program. New genes have to be discovered to make continued progress. Initial discoveries will be made for genes with the largest effect on fertility. Genes with smaller effects on fertility will be discovered later because they will be more difficult to find.

WHAT NEW TOOLS ARE NEEDED?

There will always be a need for new methods to treat anoestrus and control the oestrous cycle. The principle tools for oestrous cycle control (PGF, GnRH, oestradiol, and progesterone) were developed decades ago, and no new drugs for oestrous cycle control have been discovered in recent years. The use of progesterone and oestradiol is raising public concern about steroids in food animals. The limitations of each drug, recognised at the time of their discoveries, have not been overcome. Newer drugs with improved efficacy need to be discovered, but the economic return for new animal drugs is relatively small compared with the economic return for new human drugs. Thus, work in this area does not attract large companies capable of developing the needed products. Developing new delivery systems for existing products makes sense. Decreasing the number of times an individual animal is handled (i.e. reducing the hassle factor) will improve the uptake of a new technology. Many of the existing protocols could be simplified by mechanised methods for drug delivery.

Animals need to be inseminated so that they can become pregnant. Determining when to inseminate depends on overt signs of oestrus. Technologies that are designed to detect the overt signs of oestrus are well developed. Unfortunately, the intensity of oestrus expression may be less in modern animal production systems. Animals with subtle signs of oestrus, or animals undergoing silent oestrus (ovulation without any behavioural sign of oestrus), will not be detected. Biological monitoring of hormones may be a more effective approach, but robust methods for measuring milk progesterone in real-time (in-line test) are still in their infancy. Regardless of the detection system, any new method to increase sperm life in the female reproductive tract would greatly improve reproductive rates because time of semen deposition relative to the time of ovulation would be less critical.

Greater intensification of reproduction is associated with earlier detection of pregnancy. The value of a pregnancy test is in the identification of non-pregnant cows that need to re-enter the breeding program. An ECF test was shown to be ineffective, but the concept of identifying non-pregnant cows shortly after breeding is sound. Tests that can detect the presence of the embryo around maternal recognition of pregnancy (18-20 days after breeding) are tenable given the strong signal emitted by the embryo at that time. Cows found to be non-pregnant could be treated with PGF and GnRH, and be re-inseminated before their natural return to oestrus.

Herd sizes are increasing. Greater herd size has led to less hands-on animal management. Computers can help with animal management, but effective programs need to be developed. Reproductive management needs to be run by smart computers that are capable of making good decisions. For dairy, the tools and technology are available to let computers decide whether a cow should be scheduled for rebreeding (based on the economics of the individual cow) and when to do it. If timed AI is used, then the programming becomes simpler because treatments and inseminations can be scheduled. If electronic methods of oestrus detection are used, then the computer needs to be able to integrate the information with whatever else is known about the cow (e.g. previous oestrous dates, treatments, changes in milk production) and make decisions on the likelihood of oestrus. In either case, the system needs to be linked to automatic sort gates so that the decision that the computer makes can be enacted when the cow leaves the milking parlor. Farm workers can then implement the management (e.g. injections, inseminations, pregnancy check) on the sorted pen of cows and feed data back into the system.

All of the developments mentioned above need to be combined with new reproductive genetics. The era of single trait selection for farm animals has probably ended. Future animals will be selected for their ability to reproduce within an agricultural system. Dairy cows that make a lot of milk have more value if they also get pregnant during lactation. Traditional selection methods can make initial progress in this area. Additional gains will be made when the genes controlling reproduction are fully understood.

CONCLUSIONS

There are numerous challenges for reproductive management. Herds are getting larger and animals are more productive. Increasing reproductive rates will improve farm efficiency, but better reproduction must be achieved with a minimal expense or a large return on the investment. Genetic approaches to improved reproduction are a logical first step. Cows with better fertility will always be valued on farms. New methods to make reproduction easier and more efficient will add to the value of a high fertility animal. These new methods will shorten the interval to conception and also decrease the time required for herd reproductive management. No single method will achieve these goals. A management strategy that employs a variety of new approaches will be required.

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