Highlights of the Past and a Look into the Future of Dairy Reproduction

Matthew C. Lucy

Division of Animal Sciences, University of Missouri, Columbia 65211
Email: lucym@missouri.edu

Take Home Messages

- Highlights from the past 100 years include: artificial insemination, estrous synchronization, pregnancy diagnosis, genetic selection for fertility, and embryo transfer.
- Research in the next 100 years will focus on improving existing technological highlights so that they are more effective, less expensive, and easier to use on large farms.
- Genetic selection for fertility will lead to high milk producing dairy cows with superior fertility.
- Now is the time to define "high fertility". Cows that require hormonal interventions in order to become pregnant should not be scored as "high fertility" within genetic selection programs.

Introduction

The past 100 years in dairy reproduction has been an era of change. The birth of artificial insemination (AI) within the dairy industry occurred about 75 years ago (Foote, 2002). According to MacLeod (2009) "Good ideas have lonely childhoods". This was certainly true of AI as this new technology was viewed with skepticism initially because it was "unnatural" and faced seemingly insurmountable technical hurdles to its widespread implementation. What we found out, of course, was that attitudes can change and that hurdles can be overcome so that a seemingly fantastical new technology can become commonplace in the dairy industry. This sequence of events (initial skepticism and rejection followed by acceptance and finally widespread implementation) is common for successful new ideas and new technology in dairy reproduction. The future will be the same as new technology is tested and decisions about its commercial utility are made. If the next 100 years are as innovative as the past 100 years then the future of dairy reproduction is very promising.

WCDS Advances in Dairy Technology (2012) Volume 24: 17-28

▪ Highlights from the Past

In the author's opinion, the five highlights from the past 100 years are: 1) the development of methods and the commercial implementation of artificial insemination (AI); 2) the development of products and programs for estrous synchronization and timed AI; 3) Ultrasound-based and PAG (blood)-based pregnancy diagnosis; 4) the advent of genetic testing and genetic selection for fertility in dairy cattle; and 5) development of methodologies for embryo collection, transfer, freezing, manipulation, in vitro production, and cloning.

Artificial Insemination and Associated Technologies

Few would argue that the most significant innovation in dairy reproduction in the past 100 years is the development of methods to collect, dilute, freeze, store, and thaw semen from bulls and then use the semen to artificially inseminate cows. Also innovative was the development of dilution media for "fresh" semen that can be stored at room temperature for several days. Artificial insemination eliminated the need to keep dangerous, potentially infertile and genetically dubious dairy bulls on the farm. The bulls were replaced with a product with proven safety, excellent fertility, and known (superior) genetics. The methods used in the practice of AI (collection, dilution, freezing, and thawing of semen) have been perfected over the years. The focus has always been and continues to be on the production of a high fertility and genetically superior product. The history of AI has been reviewed (Foote, 2002).

Sexing Mammalian Sperm

A major innovation within the AI industry occurred in the 1990's when flow cytometry was used to sort X- and Y-bearing sperm (Johnson, 1995). This new technology enabled a 90% bias in the sex ratio. Despite lesser fertility of sorted sperm, this new innovation was rapidly adopted by the dairy industry to quickly expand the number of heifers arising from AI.

Devices Used to Aid in the Detection of Estrus

The innovation of AI meant that people (and not bulls) had to determine when a cow was in estrus. This was not necessarily a difficult task but it did take considerable time (a minimum of twice daily detection recommended). The need to increase efficiency in the detection of estrus has driven the commercialization of a variety of products specifically designed to identify cows in estrus. The traditional Kamar Heatmount Detector (Zionsville, IN) was introduced in 1960 and continues to be a staple for estrus detection on many dairies. An electronic heat detection patch (Heatwatch System, CowChips LLC, Manalapan, NJ) that could measure the number and duration of mounts was introduced in the 1990's but has struggled to achieve widespread application perhaps because the electronic patches are expensive and may be lost over time. Pedometers and activity monitors have surged and receded in terms of popularity over the years (Nebel et al., 2000). Their recent surge in popularity will be discussed in a subsequent section on the future of dairy reproduction.

The Development of Products and Programs for Estrous Synchronization and Timed AI

Three products are widely used in estrous synchronization and timed AI programs: prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), gonadotropin releasing hormone (GnRH) and progesterone. The development of these three products over the past 50 years is clearly the second greatest innovation in dairy reproduction. Estradiol and its derivatives were extensively used in the past because of their low cost and effectiveness but concerns over human food safety have diminished the use of estradiol products in dairy reproduction.

Products Used in Dairy Reproduction

Most of the products that are used to manage reproduction in dairy cattle had their research origins in the middle of the last century with regulatory approval thereafter. The history of the development and commercialization of products for estrous synchronization and timed AI has been reviewed (Lauderdale, 2009).

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$),

The desire to reduce the time required for heat detection led to the development and commercialization of $PGF_{2\alpha}$ (and derivatives) for the synchronization of estrus. The original work for regulatory approval was done in the 1970's. Typical programs involved a single or multiple (11 to 14 day interval) injections and insemination of cows or heifers observed in estrus. This application was approved in the United States in 1979. Two injection programs were popular in both cows and heifers and remain popular in heifers today. The two injection system forms the basis for presynchronization in Ovsynch programs (see below).

Gonadotropin-releasing hormone (GnRH)

Gonadotropin-releasing hormone causes the release of LH from the pituitary. The LH release will cause ovulation. GnRH was originally developed for the treatment of ovarian follicular cysts and continues to be used for that purpose. Studies were done in the 1970's and the first product (Cystorelin; Merial, Athens GA, USA) was approved for the treatment of cystic ovaries in 1986.

The use of GnRH within programs for timed AI has supplanted its use as a drug used to treat cystic ovaries in dairy cattle.

Progesterone

Progesterone was first used to synchronize estrus in heifers in 1948 by Christian and Casida (1948). Effective progesterone-based programs, however, were not developed until after the commercialization of $PGF_{2\alpha}$. The combination of approximately one week of progesterone and then $PGF_{2\alpha}$ treatment was an effective estrous synchronization program for many years. The development of easy-to-use intravaginal progesterone-releasing devices (CIDR or PRID) was a key innovation that enabled the safe and effective application of progesterone to dairy cattle. Progesterone releasing devices are popular today.

Programs for Timed AI

The size of dairy farms grew as the industry capitalized on economies of scale. Larger dairies typically employed fewer employees per cow. More cows meant that only those cows that expressed obvious signs of estrus were detected. The subtler behavioural signs that perhaps would be noticed on a small (60 cow) dairy were not noticed. At the same time, greater milk production per cow suppressed the expression of estrus (Wiltbank et al., 2006). These factors combined to press researchers toward developing methods for timed AI.

Ovsynch and Presynch-Ovsynch

The first timed AI program, now legendary within the dairy industry, was Ovsynch (Pursley et al., 1997). The Ovsynch program involved a series of injections (GnRH, $PGF_{2\alpha}$, and GnRH) that synchronized ovulation so that AI could be performed at a predetermined time. Ovsynch probably would not have achieved widespread application in the dairy industry had it not been for the subsequent development of presynchronization (“presynch”) programs that were administered before the Ovsynch. These presynch programs typically involve a series of $PGF_{2\alpha}$ injections to ensure that cows start on the Ovsynch program between day 5 and 10 of the estrous cycle (optimal time; Thatcher et al., 2002). They serve a second important purpose in that cows that come into estrus in response to the presynch $PGF_{2\alpha}$ injections can be inseminated with good fertility. The inseminated cows do not need to continue with the remaining injections of the Ovsynch program. Time and expense are therefore saved. There are numerous variations of timed AI programs that are popular in the United States (Presynch-Ovsynch, G6G, and double Ovsynch).

Resynch

Presynch-Ovsynch is often used for first insemination. Inseminated cows are observed for return to estrus and reinseminated when they are observed in estrus (assumed to be not pregnant). Non-pregnant cows will theoretically come back into estrus about 3 weeks after first insemination. Problems with estrous expression and the detection of estrus led to a large number of non-pregnant cows at pregnancy diagnosis (35 to 40 days after first insemination). “Resynch” involves using Ovsynch on cows that are diagnosed not-pregnant. Applying Ovsynch (first insemination) followed by resynch (subsequent inseminations) is a management strategy that is used to shorten the time between AI.

Ultrasound-based and PAG (blood)-based Pregnancy Diagnosis

Although still very popular, the traditional method of manual palpation of the uterus through the rectal wall (rectal palpation) is gradually being replaced by alternative methods. This is particularly true for early pregnancy diagnosis where dairymen desire an earlier identification of cows that are not pregnant after AI. This earlier diagnosis of non-pregnant cows enables an earlier intervention and reduces the overall time to pregnancy.

Ultrasound-based Pregnancy Diagnosis

Ultrasound had its roots in dairy reproduction as a research tool for studying ovarian follicular waves and the corpus luteum. It was then applied commercially for on farm pregnancy diagnosis (Fricke, 2002). The original ultrasounds were large, poorly portable and could not be used outdoors because the video screen was difficult to see. Modern ultrasounds are entirely portable with battery power and are typically equipped with goggles so that the examiner can see the image inside or outside the barn. Ultrasound pregnancy diagnosis has improved the accuracy of pregnancy detection for early pregnancies (less than 35 days) and is typically used for cows that are 30 or greater days pregnant. At later times (70 to 90 days) ultrasound can be used for sexing the fetus.

PAG-based Pregnancy Diagnosis

Palpation and ultrasound examination for pregnancy require technical skill. An innovative alternative is the blood pregnancy test. There are three viable commercial tests [BioPRYN (BioTracking, Moscow, ID USA), IDEXX (Westbrook, ME USA) and DG29 (Conception, Beaumont, QC Canada)]. Each test is in an ELISA format and detects pregnancy-associated glycoproteins (PAG) in blood. The blood pregnancy tests can be applied slightly earlier than ultrasound (day 25 of pregnancy or later).

Genetic Evaluation, Testing and Selection for Fertility in Dairy Cattle

Genetic selection in dairy cattle has traditionally been done for milk production. One reproduction lowlight was that the dairy industry failed to include reproduction in their selection indices until reproduction had declined to unacceptable levels. This changed in the mid-1990's when the Net Merit Index included the non-production trait – productive life. The introduction of productive life had an immediate effect on fertility because most cows that left the herd early did so because they were not pregnant. Subsequently, the trait "Daughter Pregnancy Rate" (DPR) was developed and introduced into Net Merit in 2003. The development and implementation of DPR was an important genetic innovation for the dairy industry. A second important genetic innovation was the introduction of a fertility index for bulls (ERCR or SCR; see below). The SCR trait enabled dairy producers, for the first time, to identify bulls with superior fertility and use them to improve herd reproduction.

Daughter Pregnancy Rate (DPR)

The DPR is a function of days open. One unit of DPR is equivalent to 4 fewer days open. The trait is easy to score because the only information required are two consecutive calving dates (this interval minus gestation is equal to the days open that can be converted to DPR). The DPR has been included in selection indices and the inclusion of this trait along with the inclusion of productive life has been credited with reversing the decline in fertility of dairy cows. Genetic data demonstrate an improvement in dairy cow fertility over the past 10 years.

ERCR and SCR

The renewed emphasis on all aspects of dairy fertility led to the genetic evaluation of AI sires for their fertility. The original trait "ERCR" (Estimated Relative Conception Rate) was replaced by "SCR" (Sire Conception Rate) in 2008. For SCR, a sire with an SCR of 2%, for example, would be expected to have a conception rate within the herd that is 2% above the average conception rate for the herd (i.e., 32% compared with 30% in this example).

Development of Embryo Collection, Transfer, Manipulation, in vitro Production, and Cloning Methodologies

The first successful embryo transfer in a cow was done in 1950 by Elwyn Willett and his colleagues at the American Foundation for the Study of Genetics located near Madison, Wisconsin (Betteridge, 1999). Since that historic time, the technical achievements of superovulation, nonsurgical embryo flushing, embryo freezing, embryo manipulation (splitting, etc.) in vitro embryo production, and cloning (somatic cell or otherwise) have been achieved. These technologies have been actively applied by those attempting to improve the underlying genetics of dairy cattle typically by focusing on superior females. The status of the embryo transfer industry was extensively reviewed about 10 years ago (Hasler, 2003).

▪ A Look into the Future of Dairy Reproduction

Predicting what will happen in the next 100 years is nearly impossible. Nonetheless, it is an interesting exercise and will make for good reading 100 years from now! The author believes that it is safe to assume that there will be continued pressure on milk production per cow. The carbon footprint per unit of milk of a high production dairy cow is less than that of a low production cow (Capper et al., 2009). This fact along with the need to produce more food per unit land area will drive milk production per cow to greater levels. More milk production per cow will further stress the reproductive system of dairy cattle.

Old Technology with Room for Improvement

Perhaps an easy way to discuss the future is to examine the past highlights and assess where these highlights can be improved upon.

Potential Improvements to Artificial Insemination (AI)

Artificial insemination is a tremendous technological success story for the dairy industry. There are, nonetheless, several “holy grails of AI” that presumably will be attained in the next 100 years. First, there is the need to increase sperm production per bull or to develop methods to dilute semen further without compromising fertility. A two-fold dilution, for example, would double the number of units coming from an elite sire. The second holy grail is to increase the lifespan of bovine sperm in the female reproductive tract. This would greatly facilitate the use of AI because cows could be inseminated long before ovulation without a decrease in fertility. Third, liquid nitrogen and a liquid nitrogen tank provide safe storage of semen for an indefinite period of time. A simpler and effective method for long term storage of semen on farm, however, would certainly be a welcome innovation.

Better Methods for Gender Selection of Semen

The usefulness of gender selected semen within the dairy industry has been demonstrated but the current method to generate the product (flow cytometry) is extremely inefficient. So much so that sexing is typically not used on elite sires. New methods to sex semen are clearly needed and will theoretically be achievable in the next century.

Back to Estrus

There is the possibility that consumers will increase their control over methods used in dairy production and force currently legal practices out of existence. This was recently done when recombinant bovine somatotropin (rbST), a federally approved and legal animal drug, was forced out of the marketplace in many regions when concerns over the healthfulness of milk arose. Estradiol cypionate was removed from the market in the United States because of potential consumer concerns about its use in lactating cows. Similar concerns, founded or otherwise, could arise for $PGF_{2\alpha}$, GnRH, and progesterone particularly when they are blanket applied in timed AI programs.

Farmers detect cows in estrus because estrus is the best predictor of ovulation. Timed AI programs are used because they do essentially the same thing (enable farmers to know when ovulation will occur). If timed AI programs are forced out of existence then farmers will need to detect cows in estrus or, alternatively, be able to predict when they will ovulate.

It is very unlikely that we will return to the recommendation of twice-daily 30 minute observation of estrus. The time (30 minutes) for estrus detection is too short and herds are too large to achieve efficient reproduction using this approach. Automated methods for detection of estrus, therefore, will come of age in the next 100 years. Surprisingly, the requirements for automated estrous detection systems have not changed a lot since they were first proposed by Senger (1994). Specifically there is a need for: 1) continuous surveillance of the cow; 2) accurate and automatic identification of the cow; 3) lifetime operation; 4) minimal labour; and 5) high accuracy in identifying the physiologic or behavioural events that correlate with ovulation. This author will add that the system needs to be inexpensive and seamlessly integrated into the herd management software.

Automated systems for activity monitoring meet many of these criteria and, indeed, within the United States there has been of flood of new systems introduced in the past 5 years. Although the new monitors are technologically superior to the old systems, they essentially rely on a premise that has been in existence for a long time (cows in estrus are more active). High production cows, however, are less active compared with low production cows in estrus (Wiltbank et al., 2006). This fact may blunt the utility of these programs in the next century.

Automated milk progesterone systems (DeLaval Herd Navigator System, for example) provide an interesting new alternative for dairy producers. Estrus is preceded by a drop in milk progesterone concentrations. By monitoring milk progesterone, therefore, it is possible to predict (approximately) when a cow will be in estrus. The question is whether this approximation will be good enough for routine AI. In addition to potential application for timing of AI, milk progesterone systems offer a unique opportunity to perform pregnancy diagnosis based on sustained elevated concentrations of progesterone.

New Products for Estrous Synchronization and Timed AI

The concepts and products that we currently use for estrous synchronization ($PGF_{2\alpha}$, GnRH, and progesterone) in some cases outdate the discovery of the DNA double helix (1953). Each of these products has challenges that will theoretically be overcome in the next 100 years. For example, $PGF_{2\alpha}$ does not regress the early cycle corpus luteum. The challenge can be over-come somewhat by doubling the dose or alternatively giving two injections at a 24 hour interval but perhaps there is an easier way to increase the efficacy of $PGF_{2\alpha}$. Likewise, GnRH does not efficiently cause ovulation during the mid-luteal phase. This fact limits its utility in some programs. Luteinizing hormone would theoretically be superior to GnRH but, surprisingly, recombinant bovine LH is not available for use in dairy cattle. A surrogate product (hCG isolated from human placenta) is used. It is very likely that recombinant forms of both LH and FSH will be available to the dairy industry in the near future. The recombinant LH will replace GnRH for synchronizing a follicular wave and inducing ovulation during timed AI. The recombinant FSH should improve superovulatory responses over the currently used porcine pituitary preparation. Finally progesterone encased in silicone within an intravaginal implant is a simple and effective method of delivery but the steroid residue remaining in the device after use is far too great and the delivery of progesterone from the device is too low for large metabolically active dairy cows. Better methods to delivery progesterone to the cow (electronic pumps, etc.) will be developed in the future.

New Methods for Pregnancy Diagnosis

If a cow is not pregnant to an insemination then the shortest interval from first insemination to second insemination is always best economically (Cabrera et al., 2011). Most inseminated cows have a fertilized embryo after breeding but nearly 50% of those cows lose the embryo by day 7. If these non-pregnant cows are not observed in a return estrus then approximately 4 weeks are lost from day 7 to pregnancy diagnosis (day 35) and additional time is lost when cows are resynchronized for AI. If pregnancy could be diagnosed on day 7 then this would save at least 4 weeks for a large percentage of inseminated cows. Genes are up-regulated during maternal recognition of pregnancy in cattle (day 16 to 20; Green et al., 2010) and specific genes (Isg15, Oas1, and Mx2) can be used to detect pregnancy within the first 3 weeks after AI. The challenge is that the gene-based pregnancy test is difficult to do in the laboratory (requires at least 8 hours). If a simple test could be developed then testing cows for pregnancy within 16 days after AI is possible given today’s knowledge. A realistic goal for the future will be to develop a pregnancy test that can be applied within the first week after AI. Regardless of when it is applied, an ideal pregnancy test would be usable on the farm and could be completed rapidly alongside the cow. This would enable efficient management of non-pregnant cattle.

Genetic Testing and Genetic Selection for Fertility in Dairy Cattle

The original human genome sequencing required over 10 years to complete and cost approximately 3 billion dollars. Innovations in genome sequencing methods have since led to the fast and inexpensive sequencing of entire genomes of diverse organisms. The cow genome sequence was completed in 2009 and this sequence is being used to develop genome-based methods to select for improved fertility. Genome-based selection will rapidly improve the fertility of dairy cows in the future. If genetic progress in fertility continues at the current pace then high producing dairy cows will be highly fertile within the next 100 years!

Selecting dairy cows for improved fertility is a good thing and genetic selection is a powerful tool. Genetic selection as it is practiced today (genomic selection or otherwise), however, is a black box. The physiological mechanisms that lead to improved fertility are not important. The only important outcome is the improvement in fertility.

Now is the Time to Define "High Fertility"

Given the black box of the genetic selection process, it is extremely important that the dairy industry define "high fertility" and adopt a genetic selection program (no matter how difficult) that yields the desired "high fertility" cow. The current fertility trait (DPR) is a function of days open (interval from calving to pregnancy). For dairy systems that do not intervene with reproduction (i.e., do not use $PGF_{2\alpha}$, GnRH, and (or) progesterone treatment at any time and do not use timed AI) then DPR is an excellent fertility trait. Cows that are cycling, come into estrus, are observed in estrus (intense signs), are inseminated and become pregnant during the early postpartum period have the most desirable DPR. They are highly fertile because their reproductive system is fully functional. Cows that are not cycling or do not come into estrus (silent ovulation) or have subtle signs of estrus or do not become pregnant after insemination will have longer days open and a poor DPR. They are infertile because their reproductive system does not function when placed under lactation stress. The concern is that timed AI programs mask infertility by enabling otherwise infertile cows (non-cyclers, etc.) to become pregnant. These infertile cows may have a desirable DPR because they were timed AI and become pregnant early postpartum but their reproductive system does not function well independent of external intervention. If a genetic recording system is not developed that can account for the breeding interventions used in postpartum cows then we may find that 100 years from now our population of "fertile" cows are only "fertile" when treated with $PGF_{2\alpha}$, GnRH, and (or) progesterone and timed AI. The abrupt disappearance of synchronization programs could trigger another reproductive crisis in dairy cows.

But what about the dairymen that want a cow that responds well to timed AI? We know that cows that are cycling, come into estrus, are inseminated and become pregnant during the early postpartum period perform well in timed AI programs. Selecting cows for a functional reproductive tract is the best way to select cows that will respond well to timed AI programs.

Development of Embryo Collection, Transfer, Manipulation, in vitro Production, and Cloning Methodologies.

Hasler (2003) summarized emerging new technology within the embryo transfer industry. This article was written about 10 years ago and some of the technical challenges have already been achieved! There continues to be a need for improvements in superovulation (number of embryos produced per cow). In vitro production of embryos is possible but in vitro produced embryos do not freeze well and are not equal in quality when compared with a fresh in vivo produced embryo. If commercial scale production of high-quality, low-price frozen embryos can be achieved then this would revolutionize dairy reproduction particularly during the summer when embryo transfer is superior to AI in terms of pregnancy rates. Although cloned and transgenic embryos have tremendous promise in future agricultural systems their fate may lie with consumers who will decide if they want to consume food from this type of animal.

■ Summary

There have been many highlights from the past 100 years in dairy reproduction. The author is confident that the next 100 years will produce additional highlights that go beyond our imagination. There are many reasons to believe that genetic selection for fertility will lead to high milk producing dairy cows with high fertility. Now is the time to define “high fertility” within our genetic selection programs so that the next 100 years of genetic selection yields a high milk producing dairy cow with a fully functional reproductive system that operates with no external intervention whatsoever.

■ References

Betteridge, K. J. 2000. Reflections on the golden anniversary of the first embryo transfer to produce a calf. Theriogenology 53:3-10.

Cabrera, V. E., J. O. Giordano, and P. M. Fricke. Economics of resynchronization with chemical tests to identify nonpregnant cows. *In:* Proceedings of the 2011 Dairy Cattle Reproduction Council Kansas City, Missouri. pp 57-67.

Capper, J. L., R. A. Cady, and D. E. Bauman. 2009. The environmental impact of dairy production: 1944 compared with 2007. J. Anim. Sci. 87:2160-2167.

Christian, R. E., and L. E. Casida. 1948. The effects of progesterone in altering the estrous cycle of the cow. J. Anim. Sci. 7:540 (Abstr.).

Foote, R. H. 2002. The history of artificial insemination: Selected notes and notables. J. Anim. Sci. 80 (E Suppl 2):1-10.

Fricke, P. M. 2002. Scanning the future--ultrasonography as a reproductive management tool for dairy cattle. J. Dairy Sci. 85:1918-1926.

Green, J. C., C. S. Okamura, S. E. Poock, and M. C. Lucy. 2010. Measurement of interferon-tau (IFN-tau) stimulated gene expression in blood leukocytes for pregnancy diagnosis within 18-20d after insemination in dairy cattle. Anim. Reprod. Sci. 121:24-33.

Hasler, J. F. 2003. The current status and future of commercial embryo transfer in cattle. Anim. Reprod. Sci. 79:245-264.

Johnson, L. A. 1995. Sex preselection by flow cytometric separation of X and Ychromosome-bearing sperm based on DNA difference: a review. Reprod. Fertil. Devel. 7:893-903.

Lauderdale, J. W. 2009. ASAS centennial paper: Contributions in the Journal of Animal Science to the development of protocols for breeding management of cattle through synchronization of estrus and ovulation. J. Anim. Sci. 87:801-812.

MacLeod, H. 2009. “Ignore Everybody: and 39 Other Keys to Creativity” Penguin Books, London England.

Nebel, R. L., M. G. Dransfield, S. M. Jobst, and J. H. Bame. 2000. Automated electronic systems for the detection of oestrus and timing of AI in cattle. Anim. Reprod. Sci. 60-61:713-723.

Pursley, J. R., M. R. Kosorok, and M. C. Wiltbank. 1997. Reproductive management of lactating dairy cows using synchronization of ovulation. J. Dairy Sci. 80: 301-306.

Senger, P. L. 1994. The estrus detection problem: new concepts, technologies, and possibilities. J. Dairy Sci. 77:2745-2753.

Thatcher, W. W., F. Moreira, S. M. Pancarci, J. A. Bartolome, and J. E. Santos. 2002. Strategies to optimize reproductive efficiency by regulation of ovarian function. Domest. Anim. Endocrinol. 23:243-254.

Wiltbank, M., H. Lopez, R. Sartori, S. Sangsritavong, and A. Gümen. 2006. Changes in reproductive physiology of lactating dairy cows due to elevated steroid metabolism. Theriogenology 65:17-29.