The Future of Reproductive Management

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Introduction

To fulfill the increasing requirements of the world population for dairy products, it is essential to adopt management practices and technologies that improve production efficiency, while reducing use of resources and lessen the environmental impact. Continued genetic progress for milk production coupled with nutritional management of high producing dairy cows, without attention to reproductive performance, has contributed to an inverse relationship between milk production and reproduction. Rodriguez-Martinez et al. (2008, 2012, 2013), in extensive and critical reviews, identified low fertility with the following series of factors: insufficient emphasis within genetic selection programs for longevity, health and fertility; interrelated factors such as negative energy balance (NEB), level of milk production, dystocia, retained fetal membranes, twinning, stillbirths, and endometritis; as well as inadequate attention to body condition, nutrition, reproductive management, infectious diseases, animal comfort, and housing.

Presently, research and extension efforts focus on programs of reproduction management that transcend reproductive processes and coordinate the disciplines of physiology, nutrition, health, cow management and welfare, and genetics. The dairy producer of today has a repertoire of approaches to coordinate the needs of the high producing dairy cow in order to reproduce in an efficient manner. Indeed a holistic approach to reproductive management is available now and will be further refined via continued advancements in technology. A testimony to the need for such an approach is evident when examining the current program agenda for DCRC 2013, which touches on a plethora of diverse systems to be coordinated in order to achieve pregnancy.

Advances in reproductive technologies and genomics offer wider use of germplasm. Technologies associated with sexed sperm, timed artificial insemination (TAI), and pregnancy diagnosis offer the potential to selectively enhance the impact of superior animals on production of food for human consumption. Detailed studies of tissue and cell biology, utilizing the techniques of genomics, proteomics, and bioinformatics will undoubtedly allow investigators to understand the limitations to efficient reproductive processes and provide new windows of opportunity to enhance herd fertility. Such technological advancements coupled with targeted use of nutraceuticals and careful management of critical biological windows will improve both milk production and reproductive efficiency. It is essential is to have these technological approaches transferred into the production system. Currently successful producers are insightful and ready adopters provided that they have confidence in the technology and the likelihood of improved profitability. Adoption decisions and profitability of advanced breeding technologies have been analyzed for U.S. dairy farms (Khanal and Gillespie, 2010). Farms adopting AI and embryo transfer and/or sexed sperm are, in general, managed by relatively younger and more educated producers who do not work off-farm and plan to continue farming for at least 10 years into the future. They also produce more milk per cow than non-adopters. The objective of this paper is to present a perspective of current and future advancements in reproductive management that will improve efficiency of production, health and welfare of dairy cows.

OPTIMIZATION OF REPRODUCTIVE PROGRAMS FOR TIMED ARTIFICIAL INSEMINATION (TAI)

It is clear that the duration of estrus is reduced as milk production increases, and the frequency of double ovulations and subsequent occurrence of twins also increases in cows with greater milk production at the time of the breeding period. The high producing dairy cow of today expresses estrus for approximately 7 hours during which time an average of 6.5 standing events takes place with an accumulative period of standing of 20 seconds (i.e., 3 seconds per standing event; Lopez et al., 2004). Consequently, means to increase heat detection accuracy and efficiency will be helpful in improving herd reproductive activity.

The latest versions of electronic heat detection accurately measures cow movements using neck-mounted activity tags containing a microprocessor and a three-dimensional accelerometer, or electronic recordings of pedometer activities. The activity tag monitors specific heat-related movement and its intensity, resulting in heat detection accuracies of 90 to 95 percent (Stevenson Jeff, http://www.hoards.com/E_reproduction/REP31). Increasing heat detection rates result in greater AI submission rates and more potential pregnancies. They will continue to become an important tool of electronic monitoring for reproductive activity, cow well-being, etc. and will be further addressed later on in this presentation. Nevertheless, the use of this technology will not necessarily address the issues of cattle infertility associated with anovulation, cystic ovaries, and ovarian and uterine incompatibilities associated with metabolic challenges of high milk production.

It is essential that dairy producers, farm staff, nutritionists, and veterinarians understand the physiological underlying reasons why certain components of the reproductive management program are able to improve reproductive performance or conversely why a misunderstanding of the program can lead to catastrophic pregnancy results. No one reproductive breeding program is practical and economically optimal for all dairy production units due to differences in herd management, available facilities, size of the dairy, labor that places reproduction as a high priority, and a functionally dynamic record system. What is essential is the implementation of optimal programs that fit the ongoing management system of the dairy operation and that are economical to meet the goals of the dairy (Ribeiro et al., 2012b). These optimized programs have become fertility programs that achieve more than just inseminating all cows.

Since development of the original Ovsynch TAI program, various physiologically-based modifications have been implemented to further enhance herd fertility and pregnancy/TAI when used in either intensive (Herlihy et al., 2012; Santos et al., 2010b) or grazing (Riberio et al., 2012a) dairy systems. The two most advanced systems are the Presynch-5 day Cosynch and the Double Ovsynch TAI programs (Figure 1). The two programs regulate the reproductive system to enhance fertility of the subfertile lactating dairy cowby reducing follicle dominance and sustaining a progesterone balance between injections of GnRH and PGF, by enhancing complete CL regression prior to 2nd GnRH, and by optimizing TAI relative to injection of GnRH. Programming the stage of the estrous cycle to early diestrus (e.g., days 5-9 of the estrous cycle) when the modified Ovsynch protocols are implemented has integrated multiple effects; specifically, increased probability that 1) the first injection of GnRH will induce ovulation of a first wave follicle and subsequent recruitment of a new follicle wave; 2) adequate luteal phase progesterone concentrations exist throughout the period between the first injection of GnRH and injection of PGF, 3) a CL is present to respond to the luteolytic injection of PGF, 4) the

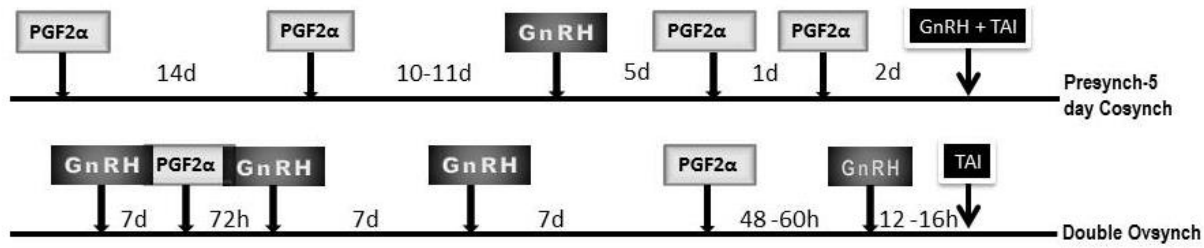

Figure 1. Diagram of the Presynch-5d Cosynch and Double-Ovsynch programs used for optimizing timed artificial insemination (TAI) in lactating dairy cows with the timely injections of GnRH and $PGF_{2\alpha}$.

process of luteolysis is complete at the time of GnRH and/ or TAI, 5) a viable oocyte is programmed for proper timing of fertilization, and 6) there is subsequent development of a robust CL to sustain a pregnancy. Indeed GnRH-induced ovulation of the first follicle wave results in the presence of both the original CL and an accessory CL at the time of PGF injection.

These two systems most definitely enhance pregnancy per TAI and provide producers alternatives that best fit their management systems. Head to head comparisons of the Presynch-7 day Ovsynch and the Double Ovsynch TAI programs gave comparable pregnancy/TAI results in multiparous dairy cows (Herlihy et al., 2012).Indeed pregnancy per AI was greater in primiparous cows enrolled in a Double Ovsynch program most likely due to inducing cyclicity and pregnancy in anovulatory cows. Comparison of a Presynch-5 day Cosynch and a Double Ovsynch protocol that incorporated a 5-day Cosynch gave comparable pregnancy/TAI results and no response differences were detected between primiparous and multiparous cows managed n a grazing system (Ribeiro et al., 2012a).

Producers that choose to incorporate an estrous detection system with AI have an opportunity within the Presynch-5 day Cosynch system to inseminate following the second PGF injection of presynchronization prior to the modified Ovsynch protocol. The beneficial effect of reducing follicle dominance is captured in the Presynch-5 day Cosynch protocol due to a reduction in the interval between GnRH and PGF injections from 7 to 5 days. However, it is essential to inject PGF twice (e.g., days 5 and 6; Figure 1) to insure complete regression of the CL, which also can further enhance pregnancy per TAI (Ribeiro et al., 2012c; Santos et al., 2010b). In the Presynch-5 day Cosynch program, timing of follicle growth is such that GnRH and TAI can be done concurrently with no reduction in pregnancy/TAI (Bisinotto et al., 2010). The use of the 5-day Cosynch program in dairy heifers is quite efficient reaching 60% pregnancy/TAI (Lima et al., 2013).

Timed AI protocols have been developed to resynchronize cows that underwent TAI but were diagnosed as non-pregnant to the first TIA. Following a first TAI with the Presynch-5 day Cosync, which obtained a 45.9% pregnancy rate at day 32/TAI, the cows diagnosed as nonpregnant began a 5 day Cosynch program at day 34 after the previous TAI either with or without an intravaginal insert containing 1.38 g of progesterone (Eazi-Breed CIDR Cattle Insert, Pfizer Animal Health, New York, NY; Bisinotto et al., 2012). Cows supplemented with progesterone had greater pregnancy/AI compared with unsupplemented cows (51.3 vs. 43.1%). Premature ovulation tended to be greater for controls compared with progesterone-treated cows (7.5 vs. 3.6%). A resynchronization for second service was tested utilizing a Double Ovsynch program beginning at 22 days after first insemination and was compared to an Ovsynch program. The breeding Ovsynch component of the Double Ovsynch and the control Ovsynch treatment group both began at day 32 in nonpregnant lactating cows (Giordano et al., 2012). The Double

Ovsynch resynchronization program increased fertility (37.3 vs. 27%). This was attributed primarily to an increase in synchronization of cows during the Ovsynch component of the Double Ovsynch protocol before TAI. These advancements allow on-farm pregnancy rates of 40 to 50% for first and second service. However, they require maximal compliance in protocol implementation, integration with on-farm computer monitoring for lists of cows to be handled, and monitoring the efficiency of the system.

Technological strategies dealing with the transition period and postpartum health

Current improvements of herd fertility that are directly applicable today have come from an advanced understanding of the processes of follicle and CL development, CL regression, ovulation, and development of the embryo. Furthermore, the programs described have provided a platform for an awareness of a need to meet the unique metabolic and endocrine challenges encountered in lactation. They have substantiated the importance of the transition period and postpartum health as biological windows that often compromise the reproductive competence of cows entering the breeding period (Santos et al., 2010a). The dairy producer in the future needs to undertake a holistic approach to optimize herd fertility.

As cows undergo transition from the pregnant nonlactating state to lactation, homeorhesis orchestrates the mobilization of tissues, primarily adipose, to meet the needs of milk production at a time when dietary nutrient intake is not sufficient to support lactation. Thus, the cow is in a period of declining nutrient status that may begin in the last 2 wk before calving, but becomes markedly negative early in the postpartum period, with a nadir between 10 and 15 d postpartum, and a return to a positive energy balance at approximately 5 to 8 wk of lactation. This dynamic period is reflected by changes in body condition that approximates lipid stores associated with subcutaneous fat. Cows with a body condition score (BCS) < 3.00 (5-point scale) either at calving or at AI have marked reductions in pregnancy per AI at 30 and 58 d after insemination (Thatcher et al., 2011). Furthermore, the change in BCS from calving to AI also influenced fertility; cows that lost ≥ 1 BCS unit had lower pregnancy per AI and greater pregnancy loss. Leblanc et al. (2010) concluded that any association between milk yield and the probability and timing of pregnancy was not clear, either among cows with distinct differences in production, or with increasing milk production over time. This indeed implies that there are marked differences among cows in their efficiency to cope with (NEB) in the transition/postpartum period, and it is not simply high milk production per se that is influencing subsequent reproductive performance. Nevertheless, there are critical alterations in biochemical and physiological aspects associated with disorders in mineral metabolism (hypocalcemia and hypomagnesemia), immune system function (retained fetal membranes, metritis, endometritis, and mastitis), and energy metabolism (ketosis, fatty liver, subacute ruminal acidosis, anovulation).These various production disorders are interrelated and associated with poor reproductive responses

As described above, BCS alone is related to subsequent fertility, and transitional changes in energy balance influence BCS. Bicalho et al. (2009) reported a significant positive relationship between BCS and size of the digital cushion of the hoof, such that low BCS cows with thinner digital cushions are at increased risk to develop sole ulcers and white line disease (i.e., lameness). Thus, cows in poor body condition would be predisposed to an increased risk of becoming lame not necessarily that lameness causes low BCS. Nevertheless, lame cows have a decreased reproductive efficiency.

In a data base developed from seven experiments conducted at the University of Illinois, the effect of prepartum energy feeding was evaluated on subsequent reproductive performance (Cardoso et al., 2013). Cows were divided into those fed a controlled-energy (CE) diet in the prepartum period (< 100% of the NE_L requirement) compared to cows fed a high-energy (HE) diet (> 100% of the NE_L requirement). Cows that received CE diets during the last 3 wk prepartum had fewer days to a subsequent pregnancy compared to cows that consumed HE diets (157 < 167 d). This positive response may be attributable in part to an increase in NE_L intake during the first 4 wk postpartum by cows fed CE diets during the close-up period. In addition, lower BCS loss in the first 6 wk postpartum, slightly greater plasma concentrations of glucose at wk 3 postpartum, and lower incidence of peripartal diseases likely contributed to improved reproductive performance. Energy-limited cows in the prepartum period had lower liver triglyceride concentrations at wk 2 postpartum, which was associated with fewer days to pregnancy. Perhaps cow differences in transition efficiency may be associated with the ability to use mobilized NEFA as a metabolic fuel after parturition. Feeding a controlled-energy diet prepartum may contribute to this ability and can be exploited to improve health and reproductive efficiency.

Monitoring of milk composition to access metabolic and health status as related to reproductive performance

The association between Fat%:Protein% Ratio (FPR) in milk and energy balance has been well characterized in first lactation dairy cows (Buttchereit et al., 2010). The FPR is greatest in the initial lactation period when energy deficit is most pronounced. When the decline in the FPR stops, energy balance stabilizes. In the early stages of lactation the correlation between FPP and energy balance among cows were the closest r = -0.43. The explanation for this biological association is that an energy deficit leads to increased lipolysis and uptake of these fatty acids by the mammary gland. Concurrently, inadequate intake of fermentable carbohydrates for ruminal bacteria fermentation may result in reduced proliferation of ruminal bacteria and thus reduced bacterial protein. The flow of amino acids to the mammary gland is reduced and milk protein percent is decreased. As a consequence an increase of the FPR to > 1.5 is indicative of high lipolysis and is predictive of early production disorders such as ketosis, displaced abomasum, ovarian cysts, mastitis, and lameness.

The moderate positive genetic associations between FPR and interval fertility traits such as number of days to first AI and days open ranged from 0.14 to 0.28 (Negussie et al., 2013). Cows with relatively greater FPR tend to have delayed first AI because of the negative EB and subsequent mobilization of body reserves characteristic of early lactation. The milk FPR ratio is a heritable trait that ranged from 0.16 to 0.25. It’s on-farm ease of availability for temporal monitoring on a within cow basis, with new online technology in the milking parlor, suggests it to be a useful tool: to monitor energy balance of individuals or the herd group, a potential response during the early critical phases of lactation for interval fertility status responses,, and potentially useful for selection of fertility.

Online technology to precisely integrate cow biological windows to achieve reproductive potential

Future advancements to further improve efficiency of the programs will entail daily monitoring online of cows for early diagnosis of pregnancy, ovarian status and ovarian cycles, metabolic and health statuses with the use of biosensor technology within the milking parlor.

Such technology combined with optimized housing to maximize animal comfort, health, and well-being will further allow high producing lactating dairy cows to reproduce and sustain high amounts of milk production. The S.A.E AFIKIM Co. (http://www1.telemessage.com/upload/infocenter/info_images/17022009212040@Afilab.pdf) of Israel presents the Afilab system with installations in Israel, Europe, and select research stations in North America. This in-line milk sensor system combined with infra-red technology estimates fat, protein, lactose, SCC and the presence of blood in the milk, by assessing the pattern of light scatter as it passes through the milk flowing into the receiver. Furthermore, concurrent integrated systems are available to monitor cow activity via cow pedometers and/or activity tags to detect changes in activity indicative of cows being detected in estrus or possibly lame, as well as an automatic walk through system to monitor body weight. A system with different technology, Herd NavigatorTM (http://www.herdnavigator.com/pages) is an automatic on-line nanotechnology laboratory developed by DeLaval and FOSS and currently distributed both in Europe and Canada. The Herd Navigator unit is installed at the farm and uses biosensor technology (Brandt et al., 2010) to measure four hormonal/metabolic molecules: progesterone, LDH (lactate dehydrogenase), BHB (beta hydroxybutyrate), and urea. Utilizing computerized bio-models on farm can describe the physiological and metabolic status of cows leading to timely decisions. Progesterone is used to detect heat, pregnancy, cysts, anestrus, and abortion. The LDH enzyme is released in milk when cells are destroyed during inflammation in the udder. The LDH is suitable for the detection of early stages of mastitis and decreases rapidly after mastitis subsides. The ketone body, BHB, is used primarily to detect sub-clinical and clinical ketosis, and other abnormalities which impair metabolism. Urea in milk can be an indicator of over or under consumption of dietary protein in the cow's ration. These technologies exemplify the potential holistic approaches of integrating lactating dairy cow physiological and metabolic statuses to optimize reproductive performance, health, and well-being of the lactating dairy cow. An even more specific biosensor to detect pregnancy likely will be developed with the recent report (Lawson et al., 2013) that pregnancy-associated glycoproteins (PAGS) can be measured in milk at day 30, as a pregnancy with a high degree of accuracy. The capability to measure PAGS in milk at day 30 of pregnancy will complement the use of TAI resynchronization programs for the re-insemination of non-pregnant cows in a timely manner.

Coordination of nutritional and reproductive management systems

It is clear that reproductive performance of the lactating dairy cow suffers when nutrient intake is inadequate. Genetic selection for increased milk production partitions more nutrients to the mammary gland at the expense of body reserves. Higher producing dairy cows are often better eaters thus are not necessarily in greater NEB. The state of (NEB) in early lactation uncouples the somatotrophic axis, which influences follicle development, steroidogenesis and, eventually, delays first postpartum ovulation. As previously discussed, feeding less energy (< 100% of NE_L requirement) to the prepartum close-up cow, seemed to increase the cow's ability to deal with the partitioning of nutrients during the period of negative energy balance. Strategies have been proposed to overcome the shortage of nutrients postpartum. Feeding diets that promote glucose synthesis and increase blood insulin concentrations favor earlier resumption of the first postpartum ovulation but have not increased overall reproductive efficiency (Thatcher et al., 2011). Similarly, supplementing diets of dairy cows with unsaturated fatty acids (i.e., conjugated linoleic acids) that suppress milk fat synthesis reduces the energetic cost for milk production, increases milk yield (Hutchinson et al., 2012), and favors earlier ovulation

(Casaneda-Gutierrez et al., 2007), but has not altered pregnancy responses (de Veth et al., 2009; Hutchinson et al., 2012). Manipulating the prepartum diet to improve Ca^{++} homeostasis postpartum and assuring adequate length of exposure to this diet prepartum appears to be critical to postpartum fertility (DeGaris et al., 2010). During the transition and breeding periods, incorporating supplemental fats and manipulating the fatty acid profiles (i.e., omega 6 and 3 concentrations) of the fat seem to be promising strategies to improve both milk production (Greco et al., 2012) and fertility (Silvestre et al., 2011a, 2011b) of dairy cows. Future investigations are warranted to determine if the beneficial effects to fertility of feeding polyunsaturated fatty acids might be further enhanced when utilized in combination with hormonal treatments that stimulate embryo development and fertility (e.g. use of bovine GH Thatcher et al., 2011). Supplemental long chain fatty acids can act as specific nutraceuticals. Feeding omega-6 fatty acids during the early postpartum period that act in a proinflammatory mode accompanied by feeding omega-3 fatty acids during the breeding period that act in an anti-inflammatory mode have improved fertility (Silvestre et al., 2011b). Future reproductive management programs on dairies will be coordinated carefully with nutritional management during the transitional and postpartum periods.

Assisted Reproductive Technology

Superovulation and Embryo Transfer

The advent of hormonal manipulation of the reproductive cycle of the cow, inducing multiple ovulations, coupled with AI, embryo collection, and non-surgical embryo transfer allows dairy producers to obtain multiple offspring from genetically superior females by transferring their embryos into recipients of lesser genetic merit. Moreover, high genetic merit embryos can be frozen for later transfer or sale. Non-surgical embryo collection and transfer procedures, with high fertility, make this technology available to producers. These procedures can be readily modified to accommodate implementation of other technology such as ovum pick up (OPU), transfer of in vitro-produced embryos, and timed embryo transfer. A major advancement has been made with the generation of long lived single-chain recombinant dually active gonadotropin analogs of FSH and chorionic gonadotropin (CG) that will allow single injection of gonadotropin for superovulation (Adams and Boime, 2008). Hopefully, this new technology will be advanced to the market place in the near future for use by producers.

In Vitro Production (IVP) of Embryos

Progress has been made in culture conditions such that immature oocytes can now be retrieved and matured in vitro (IVM), undergo in vitro fertilization (IVF), and then cultured in vitro (IVC) to the blastocyst stage for subsequent transfer to a recipient. Oocytes isolated from ovaries obtained at the slaughterhouse are used for IVP. Alternatively, oocyte retrieval by OPU can be carried out with females at virtually any age or reproductive status including prepubertal heifers and pregnant cows. This has the potential to substantially increase the lifetime productivity of high genetic merit females and effectively reduces the generation interval.

The use of sperm from high genetic merit bulls to fertilize oocytes is a means of generating large numbers of embryos with improved genetic potential utilizing selection from both the male and female (i.e., based on genomic testing, identify cows of high genetic merit as oocyte donors). The efficient use of IVP is currently limited due to lower conception rates, as a consequence of non-optimal culture conditions compared to normal superovulation and embryo transfer. The poorer fertility of IVP embryos is further exacerbated by their poor freezability as measured by reduced pregnancy rates post-thaw compared to the use of fresh IVP embryos. An improvement in embryo culture and freezing is a current area of intensive investigation world-wide that undoubtedly will improve the viability and fertility of IVP bovine embryos in the future.

Sex sorted sperm

Altering the sex ratio in favor of heifer calves offers an advantage for the dairy industry for producing replacement heifers. Until recently the only option was to directly sex embryos. However, a method to sort sperm based on DNA content was developed (Johnson, 1995). Utilizing a DNA binding fluorescent dye, Hoechst 33342, sperm are stained and sorted on a Fluorescence Activated Cell Sorter (FACS). The bovine X-bearing sperm contain 3.8% more DNA than Y-bearing sperm, allowing their separation. Most of the leading breeding companies have sexed sperm available.

Currently, there are two drawbacks associated with sexed sperm technology. First it is a very slow process, producing only 150 to 200 straws of sexed sperm per machine in a day. The second problem with sexed sperm is lowered conception rates. Two large scale summaries indicate the pregnancy rate for Holstein heifers inseminated with sexed sperm was 80% (DeJarnette et al. 2009) and 70% (Norman et al., 2010) of that using conventional sperm. For cows, the pregnancy rate was 83% of that using conventional sperm. Despite these reductions in Pregnancy/TAI, the use of sexed sperm on dairy farms could be justified because it enhances producer's ability to efficiently obtain replacement heifers thus mitigating some of the effects of high culling rates and poor reproductive efficiency. Furthermore, AI of virgin dairy heifers with X-bearing sperm would reduce calf birth weight and therefore decrease the incidence of calving difficulty. An additional practical strategy for dairy producers is to utilize sexed sperm for an efficient first service TAI of dairy heifers followed up with conventional sperm for second service.

Further strategies for using sexed sperm to improve efficiencies and cost effectiveness are being developed. A means for improving efficiencies with sexed sperm is its use for fertilization in production of IVP embryos for transfer. On average it is recommended to use two million sexed sperm to inseminate a single heifer. In contrast, less than100,000 sperm can effectively fertilize one hundred oocytes in vitro, thus reducing the impacts of damage to sperm during sorting and low sperm numbers following sexing. Furthermore, use of sex-sorted sperm for IVP resulted in normal rates and quality of blastocyst development (Underwood et al., 2010b), as well as good pregnancy success to timed embryo transfer of sex IVP embryos (Rasmussen et al, 2013) compared to IVP embryos produced from conventional sperm. Technological advancements regarding IVP of embryos and sexed sperm have the potential to revolutionize the dairy industry as progress continues in optimizing on-farm use of these systems.

Biotechnology and reproductive management

Detailed studies of tissue and cell biology, utilizing the techniques of genomics, proteomics and bioinformatics, will undoubtedly allow investigators to understand the limitations to efficient reproductive processes of the sub-fertile lactating dairy cow. The dairy producer, scientist, and industry not only need to understand and utilize the technology but also to translate the importance of these tools to the public and consumers so these new futuristic systems can be approved and adapted.

The author has chosen to end this presentation on a futuristic outlook by providing examples of divergent ongoing research technologies for reproduction that ultimately may improve efficiency of production, health, and welfare of the on-farm dairy production unit (i.e., the cow, the herd group, farm labor) and food production. The first example is the potential control of puerperal metritis which from a holistic perspective is important to reproductive performance (Bicalho R., Cornell University, personal communication). The efficacies of three subcutaneous vaccine formulations were evaluated in preventing puerperal metritis. The vaccines contained different combinations of recombinant-produced proteins (FimH; leukotoxin; and pyolysin) and/or inactivated whole cells (*Escherichia coli*, *Fusobacterium necrophorum*, and *Trueperella pyogenes*). To evaluate vaccine efficacy, a properly designed and sensitive experiment was conducted at a commercial dairy farm utilizing 265 late pregnant heifers allocated to the three subcutaneous vaccine groups and a control group. Heifers were vaccinated twice at 230 days and 260 days of pregnancy. The vaccines were found to reduce the incidence of puerperal metritis, reduced rectal temperature at 6 ± 1 d in milk, and induced a significant increase in serum immunoglobulin G titers against all antigens. Consequently, subcutaneous vaccination with inactivated bacterial components and/or protein subunits of *E. coli*, *F. necrophorum*, and *T. pyogenes* can possibly reduce the incidence of puerperal metritis during the first lactation of dairy cows.

The second futuristic example is use of synthetic biology to develop a designer network coordinating bovine AI by ovulation-triggered release of implanted sperm cells (Kemmer et al., 2010). Dairy cows were treated in utero with gelatin capsule implants containing both human embryonic kidney cells containing a transgenic LH receptor (i.e., coupled to a cAMP-mediated CREB-triggered expression of a secreted cellulase) and sperm cells. The gelatin implants were inserted into the uterus on the 2nd day of an FSH superovulation protocol, at the time of the first prostaglandin injection which was two days prior to a LH surge induced by hCG. The sequence of activated events is the following: hCG passes through the semipermeable capsule, binds to the transgenic LH receptor on the human kidney cells, expression of secreted cellulase enzyme from the cells is increased, the gelatin capsule breaks down, and sperm cells are released into the uterine environment. The released sperm cells fertilize the oocytes over a very short period of time following ovulation, and quality blastocysts were recovered at 7 d after ovulation. Such a coordinated network system could potentially improve the efficiency of dairy cow reproductive management that is physiological, eliminates the need for estrous detection, optimizes the timing of sperm availability for capacitation and fertilization, and results in timely pregnancies. The system could be further refined to be homogenous regarding bovine cells and hormones (e.g., ovarian cells with LH receptors, LH, GnRH, etc.).

These two futuristic examples demonstrate the potential of basic research applied to practical problems and exemplifies that the research today has the potential to possibly revolutionize reproductive management for the future. What an exciting proposition for the future of dairying.

Conclusions

- Electronic monitoring increases accuracies for heat detection to 90%, but will not address issues of infertility.

- Highly effective TAI programs for first and repeated services are applicable to both fertile and sub-fertile cows improve herd reproductive performance and can be combined with electronic monitoring of heats.
- Holistic approaches to integrate reproductive management with nutrition, metabolic status, and health will constitute the breeding programs of the future.
- Controlling energy in the diet prepartum minimizes drastic changes in loss of body condition postpartum, enhances transition efficiency and improves intervals to first breeding and pregnancy.
- Biosensor technology in the milking parlor allows for individual monitoring of the cow's metabolic and reproductive statuses via monitoring milk constituents and physiological statuses. This will allow for earlier and more efficient decisions to manage nutritional and reproductive management needs of individual or groups of cows.
- Specific nutriceutical approaches through manipulation of the diet will enhance both milk production and fertility via optimization of energy intake, immune function, sensitivity of tissues (i.e., mammary gland, uterus/ovary, conceptus, and coordination of peripheral tissues [i.e., liver, adipose and muscle] during critical physiological periods).
- Advancements in assisted reproductive technology will improve: efficiency of the superovulation process; freezability and fertility of IVP embryos; utilization of sexed sperm in combination with genetically superior oocytes, from genomic superior donors, to further improve rate of genetic progress.
- On farm utilization of new and exciting technologies for holistic approaches to reproductive management will increase with ease of application, effectiveness of the responses, net economic benefit, and both support and use of such technologies by producers and consumers.

Literature Cited

Adams TE, and I. Boime. 2008. The expanding role of recombinant gonadotropins in assisted reproduction. Reprod. Dom. Anim. 43 (suppl. 2), 186–192.

Bicalho R. C., V. S. Machado , and L. S. Caixeta. 2009. Lameness in dairy cattle: A debilitating disease or a disease of debilitated cattle? A cross-sectional study of lameness prevalence and thickness of the digital cushion. J. Dairy Sci. 32:3175-3184.

Bisinotto, R.S., E.S. Ribeiro, L.T. Martins, R.S. Marsola, L.F. Greco, M.G. Favoreto, C.A. Risco, W.W. Thatcher, and J.E.P. Santos. 2010. Effect of interval between induction of ovulation and AI and supplemental progesterone for resynchronization on fertility of dairy cows subjected to a 5-d timed AI program. J. Dairy Sci.93: 5798-5808.

Brandt M., A. Haeussermann, and E. Hartung. 2010. Invited review: Technical solutions for analysis of milk constituents and abnormal milk. J. Dairy Sci. 93 :427–436.

Buttchereit N., E. Stamer, W. Junge, and G. Thaller. 2010. Evaluation of five lactation curve models fitted for fat:protein ratio of milk and daily energy balance. J. Dairy Sci. 93 :1702–1712.

Cardoso F. C., S. J. LeBlanc , M. R. Murphy, and J. K. Drackley. 2013. Prepartum nutritional strategy affects reproductive performance in dairy cows. J. Dairy Sci. 96 :5859–5871.

Castaneda-Gutierrez E, B.C. Benefield, M.J.de Veth, N.R. Santos, R.O. Gilbert, W.R. Butler, D.E. Bauman. 2007. Evaluation of the mechanism of action of conjugated linoleic acid isomers on reproduction in dairy cows. J. Dairy Sci. 90:4253–4264.

de Veth M J, D.E.Bauman, W. Koch, GE. Mann, A.M. Pfeiffer, W.R. Butler. 2009. Efficacy of conjugated linoleic acid for improving reproduction: A multi-study analysis in early-lactation dairy cows. J. Dairy Sci.92:2662–2669.

DeGaris PJ, I.J. Lean, A.R. Rabiee, C. Heuer. 2010. Effects of increasing days of exposure to prepartum transition diets on reproduction and health in dairy cows. Aust. Vet. J. 88:84-92.

DeJarnette, J.M., R.L. Nebel, and C.E. Marshall. 2009. Evaluating the success of sex-sorted semen in US dairy herds from on farm records. Theriogenology 71:49-58.

Giordano J.O., M.C. Wiltbank, J.N. Guenther, R. Pawlisch, S. Bas, A.P. Cunha, and P.M. Fricke. 2012. Increased fertility in lactating dairy cows resynchronized with Double-Ovsynch compared with Ovsynch initiated 32 d after timed artificial insemination. J. Dairy Sci. 95:639-653.

Greco, L.F., M. Garcia, B.L. Artiaga, E.K. Ganda, R.S. Bisinotto, F.S. Lima, N. Martinez, E.S. Ribeiro, A.L. Lock, W.W. Thatcher, C.R. Staples, and J.E.P. Santos. 2012. Effect of supplemental fatty acids on production responses and hepatic fatty acid composition and gene expression of dairy cows fed diets containing low concentrations of fatty acids. J. Dairy Sci. Vol. 95(Suppl. 2):103 (Abstr.).

Herlihy M.M., J.O. Giordano, A.H. Souza, H. Ayres, R.M. Ferreira, A. Keskin, A.B. Nascimento, J.n. Guenther, J.M. Gaska, S.J. Kacuba, M.A. Crowe, S.T. Butler, and M.C. Wiltbank. 2012. Presynchronization with Double-Ovsynch improves fertility at first postpartum artificial insemination in lactating dairy cows. J. Dairy Sci. 95:7003-7014.

Hutchinson I. A. , A. A. Hennessy, R. J. Dewhurst, A. C. O. Evans , P. Lonergan, and S. T. Butler. The effect of strategic supplementation with trans-10,cis-12 conjugated linoleic acid on the milk production, estrous cycle characteristics, and reproductive performance of lactating dairy cattle. J. Dairy Sci. 95 :2442–2451.

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

Kemmer C., D.A. Fluri., U. Witschi, A. Passeraub, A. Gutzwiller, and M Fussenegger 2011. A designer network coordinating bovine artificial insemination by ovulation-triggered release of implanted sperms. J. Controlled Release. 150 (2011) 23–29

Khanal A.R., and J. Gillespie J. 2010. Adoption of technology, management practices, and production systems in US milk production. J. Dairy Sci. 93:6012-6022.

Lawson B, D. Ray, K. Velek, E. Martel, R. Lin-Scott, P. McCoy, M. Tate, J. Lawrence, and W. Silvia. 2013 Evaluation of the Idexx pregnancy detection assay for milk samples. J. Anim. Sci. Vol. 91, E-Suppl. 2/J. Dairy Sci. Vol. 96, E-Suppl. p115 Abstract T332.

LeBlanc S. Assessing the association of the level of milk production with reproductive performance in dairy cattle. 2010. J Reprod. Dev. 56:S1–S7.

Lima F.S., W.S. Riberiro, R.S. Bisinotto, L.F. Greco, N. Martinez, M Amstalden, W.W. Thatcher, and J.E.P. Santos. 2013. Hormonal manipulations in the 5-day timed artificial insemination protocol to optimize estrous cycle synchrony and fertility in dairy heifers. J. Dairy Sci. 96: 1-12.

Lopez H, L.D. Satter, and M.C.Wiltbank. 2004. Relationship between level of milk production and estrous behavior of lactating dairy cows. Anim. Reprod. Sci. 81:209-223.

Negussie E., I. Strandén, and E. A. Mäntysaari. 2013. Genetic associations of test-day fat:protein ratio with milk yield, fertility, and udder health traits in Nordic Red cattle. J. Dairy Sci. 96 :1237–1250.

Norman, H.D., J.L. Hutchison, and R.H. Miller. 2010. Use of sexed semen and its effect on conception rate, calf sex, dystocia, and stillbirth of Holsteins in the United States. J. Dairy Sci. 93:3880-3890.

Rasmussen, S., J. Block, G.E. Seidel, Z. Brink, K. McSweeney, P.W. Farin, L. Bonilla, and P.J. Hansen. 2013. Pregnancy rates of lactating cows after transfer of in vitro produced embryos using X-sorted sperm. Theriogenology. 79: 453–461.

Ribeiro E.S., A.P. Monteiro, F.S. Lima, H. Ayres, R.S. Bisinotto, M. Favoreto, L.F. Greco, R.S. Marsola, W.W. Thatcher, and J.E. Santos. 2012a. Effects of presynchronization and length of proestrus on fertility of grazing dairy cows subjected to a 5-day timed artificial insemination protocol. J. Dairy Sci. 95:2513-2522.

Ribeiro E.S., K.N. Galvão, W.W. Thatcher, and J.E.P. Santos. 2012b.Economic aspects of applying reproductive technologies to dairy herds. Anim Reprod, 9:370-387.

Ribeiro ES, R.S. Bisinotto, M.G. Favoreto, L.T. Martins, R.L. Cerri, F.T. Silvestre, L.F. Greco, W.W. Thatcher , and J.E. Santos. 2012c. Fertility in dairy cows following presynchronization and administering twice the luteolytic dose of prostaglandin F(2α) as one or two injections in the 5-day timed artificial insemination protocol. Theriogenology 78:273–284.

Rodriguez-Martinez H (et al., 2008, 2012, 2013. Series of articles published online ; Reproductive performance in high-producing dairy cows: can we sustain it under current practice? In: IVIS Reviews in Veterinary Medicine, I.V.I.S. (Ed.). International Veterinary Information Service, Ithaca NY, Part 1, http://dc130.4shared.com/doc/4aMb_ZN-/preview.html ; Part 2, http://en.engormix.com/MA-dairy-cattle/genetic/articles/reproductive-performance-high-producing-t2592/103-p0.htm; Part 3, http://en.engormix.com/MA-dairy-cattle/dairy-industry/articles/reproductive-performance-high-producing-t2593/472-p0.htm

Santos J.E., Narciso C.D., Rivera F., Thatcher W.W., and Chebel R.C. 2010b. Effect of reducing the period of follicle dominance in a timed artificial insemination protocol on reproduction of dairy cows. J Dairy Sci. 93: 2976-88.

Santos JEP, R.S. Bisinotto, E.S. Ribeiro, F.S. Lima, L.F. Greco, C.R. Staples, and W.W. Thatcher. 2010a. Applying nutrition and physiology to improve reproduction in dairy cattle. In: Lucy MC, Pate JL, Smith MF Spencer TE, editors. Reproduction in Domestic Ruminants VII, Nottingham University Press, pp. 387–403.

Silvestre F.T, T.S. Carvalho, N. Francisco, J.E.Santos, C.R. Staples, T.C. Jenkins, and W.W.Thatcher. 2011a. Effects of differential supplementation of fatty acids during the peripartum and breeding periods of Holstein cows: I. Uterine and metabolic responses, reproduction, and lactation. J Dairy Sci. 94:189-204.

Silvestre F.T, T.S. Carvalho, P.C. Crawford, J.E. Santos, C.R. Staples, T. Jenkins, and W.W.Thatcher. 2011b. Effects of differential supplementation of fatty acids during the peripartum and breeding periods of Holstein cows: II. Neutrophil fatty acids and function, and acute phase proteins. J Dairy Sci. 94:2285-2301.

Thatcher W, J.E. Santos, C.R. Staples. 2011. Dietrary manipulations to improve embryonic survival in cattle. Theriogenology 76: 1619-1631.

Underwood, S.L., R. Bathgate, D.C. Pereira, A. Castro, P.C. Thomson, W.M. Maxwell, and G. Evans. 2010. Embryo production after in vitro fertilization with frozen-thawed, sex-sorted, re-frozen-thawed bull sperm. Theriogenology 73:97-102.