

# Ruminant reproduction: recent findings and future challenges, a summary

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## Introduction

This conference has provided a valuable update on knowledge of reproductive physiology, and its genetic and environmental regulation, in a variety of domesticated and wild ruminants. Researchers have begun to examine genetic factors in model systems such as maturation and capacitation of sperm, follicular selection, maturation and ovulation, and luteal function in pregnant and non-pregnant females, as well as placental development and function. New knowledge of details of these processes reinforces basic concepts of the importance of neuroendocrine regulation of testicular and ovarian function, particularly in regard to roles of steroid hormones in determining fertility. One is struck by the multiplicity of effects of key hormones and the precision of timing of their secretion required for maximum reproductive performance, especially in the lactating female.

Greater understanding of genomic, proteomic and metabolomic factors is being obtained and interpretations of "omics" data are improving. A greater number of papers included data from use of new genetic and associated "omics" technologies than ever before. The elucidation of roles of non-coding RNAs, especially micro-RNAs, is revealing detailed knowledge of regulatory steps in oocyte maturation and early embryogenesis. Despite numerous steps forward, many mysteries of mechanisms of action in both the male and the female are yet to be solved.

We've seen and heard reviews of a series of careful step-by-step analyses of significant questions in reproductive physiology and endocrinology. Sometimes the work has been done by the reviewers and their colleagues. In other cases, more extensive networks of researchers were involved, either as collaborators, building on others' work, or working independently.

## Biotechnology

The initial paper by Taylor *et al.* highlighted the economy of new technologies and rapid progress in understanding the ruminant genome, with predictions of how these methods will allow manipulation in the future. The power of newer methods was emphasized further by Sinclair *et al.* and Anthony *et al.* One statement by Taylor *et al.* was that "genetic progress in milk production is expected to double due to the decrease in generation interval ... achieved by a reduced need to progeny test young bulls..." There is a danger that this prediction might portend a further decrease in reproductive fitness of lactating dairy cows unless selection programs consider reproductive traits. However, the authors optimistically concluded that such information will "guide the engineering of transgenic animals with increased adaptation to changing production environments, disease resistance, reproductive and productive capabilities."

Bauersachs *et al.* evaluated and illustrated techniques for analyses of genomic, transcriptomic, and proteomic databases. These analyses provide opportunities for linking quantitative and

molecular genetics to expand knowledge of genetic regulation of reproductive traits. Although these databases have revealed antagonistic relationships between production and fertility at the molecular level, some alleles were found that had favorable effects on yield traits and a fertility trait.

A wide variety of actions has been ascribed to non-coding RNAs in the mouse (Tesfaye *et al.*), but relatively little is known in ruminants. This is certainly an area of fertile ground for ruminant studies. The micro RNAs are particularly exciting, as it appears that they play major roles in regulating activation of the embryonic genome (Sirard) and are altered in oocytes derived from persistent follicles (Lingenfelter *et al.* 2007), which in preliminary work (Taft, Jr. 1999) appeared to have progressed in meiosis to nuclear stage II. Current knowledge of the timing and factors involved in the maternal:embryo transition of genomic control was reviewed in detail by Sirard, who pointed to the 8-cell stage as a major point of transition. He noted that transcription factors decrease through the 8-cell stage, then increase again at the blastocyst, but some transcription factors that are only maternal in origin, do not show that recovery. Tesfaye *et al.* predicted that micro RNAs will be considerably involved in post-transcriptional gene regulation and be regulated epigenetically.

Another component of the genome recently described in more detail is endogenous retroviruses. As discussed by Spencer *et al.*, these beta retroviruses began to enter the genome millions of years ago or entered thousands of years ago, but may still be invading. Although many are non-functional, others play important biological roles. They can prevent entry of other retroviruses by receptor interference. Some are expressed specifically in uterine luminal and glandular epithelia and in trophoblast of the conceptus. These play essential roles in elongation of the conceptus and in growth and development of trophoblast and could possibly predict the type of implantation in these species. Two questions come to mind: (1) Is there any potential for contraceptive effects in animal populations by blocking those retrovirus components that are essential to placentation? (2) Could any of these retroviruses qualify for use as vectors for gene insertion?

### **Comparative data have been a prominent feature of this symposium**

Comparisons across species can be very valuable in stimulating our thinking. Data reported here provide great encouragement relative to the breadth of studies involved and the variety of species being studied. However, the multitude of data on mice give cause for concern that rodents may be substituted for food animals in too many cases for the long term good of animal agriculture. While reduced funding for food animal research has caused a search for funding from biomedical sources (Ireland *et al.* 2008), it appears to have caused a shift to use of research animals from which data may or may not be extrapolated readily to food animals.

On the other hand, much of what has been learned in food animals has derived from clues gleaned from studies of lower mammals. Clarke & Smith credited the basis for their work with gonadotropin inhibitory hormone to its discovery in the hypothalamus of an even more remote species, the quail. It will be important in the future to maintain an appropriate balance and the ruminant-oriented scientist will necessarily read and study a wide selection of non-ruminant literature in the search for clues to mechanisms.

Given the increased presentation of comparative data at this venue than at previous symposia in the series, a naive science writer in attendance might conclude that there has been great success in transgenics that led to ruminant rats, ruminant mice and ruminant pigs. I am reminded of the time when my younger son was raising cattle as a 4-H youth project. Tom reasoned that winter feeding costs (and his labor) could be reduced by transferring a gene for

hibernation into the beef cow. My own dream was that isolation of the factor that caused identical quadruplets in the armadillo might allow similar division of early zygotes in food animals if that factor were injected into the oviduct. Subsequent separation of the divided embryos should facilitate an increase in genetic efficiency of embryo transfer. Might such a gene exist in dams of identical twins?

These science fiction-type ideas might not be as farfetched as one would think when considered in light of successes in rodents with transplantation of testicular stem cells. The most exciting new finding in that area is that germ cell clumps were formed in THY1-enriched spermatogonial stem cells from the bull cultured with growth factors and bovine embryonic fibroblasts as feeder cells (Oatley). Successful transplantation of spermatogonial stem cells from superior bulls into testes of average bulls would be an alternative to AI for the beef industry, in which AI is limited by extensive conditions. Of course, such a procedure also would contribute to a loss of heterozygosity in the population.

Studies of embryonic and fetal mortality are revealing species differences in types and patterns of loss, and genetic, environmental and epigenetic factors involved in those losses. Analyses of genomic, proteomic and metabolomic information may identify opportunities for interventions to prevent these losses or overcome detrimental characteristics.

Another positive feature of this symposium is the increase in number of submitted papers. Particularly noticeable to this reviewer is a greater emphasis on male reproduction among those submitted papers. The male has been neglected in some quarters in recent years. At this meeting, several submitted papers provided detailed elaboration of concepts discussed in the review papers.

### **Characterization of the ruminant genome and its components in reproductive tissues, including placenta**

Productive efforts to characterize ruminant genomes have been reported in the whole animal, ovary and oocyte, testis and spermatozoa, oviduct, uterus, and placenta. Developmental programming by dietary manipulation is receiving increasing emphasis. Fowden *et al.* characterized environmental effects on several placental functions and effects on fetal or birth weights. Nutrition at critical times during prenatal development is clearly a major factor and produces epigenetic effects that carry over into adulthood. They described a variety of roles of the placenta and the importance of imprinted genes and IGF2 in placental development and adaptation. In reading their report, the widely used ratio of fetal weight to placental weight seems a crude measure of placental efficiency. It would appear that more precise data are obtained in studies using catheterization of fetal and maternal vessels to collect sequential samples for analyses. Would some measures of placental function lend themselves to evaluation in a manner that would yield a relative value, similar to residual feed intake?

The importance of DNA methylation in gene expression and epigenetic effects, and effects of diet on DNA methylation were emphasized by both Fowden *et al.* and Sinclair *et al.* The latter authors delineated knowledge of DNA methylation in the germline in mice and how little is known in ruminants. They introduced the term "methylome" in regard to methylation status of genes. They presented evidence of dietary regulation, effects of environmental chemicals, and epigenetic changes and raised an important question, Why are "effects of peri-conceptual nutrient restriction manifest more in male than female offspring" in both rats and sheep? They further noted a neglect of research into effects of paternal nutrition on epigenetic programming through the germline. Germane to current concerns in the human population, they showed that dietary limits to methylation in the ewe led to adult obesity in the offspring.

“Knockdown” of gene expression was used by Anthony *et al.* to assess gene function in sheep placenta. With a short-hairpin RNA targeting sheep proline-rich protein 15, a nuclear protein expressed during elongation of the conceptus, introduced into the trophoblast, the conceptus died or failed to develop by day 15 postcoitum. Mechanism of action of the gene is not known. They also discussed development and use of cultures of cattle or sheep trophoblast cell lines in assessing gene function and regulation, and limitations to *in vitro* techniques.

### Seasonal and neuroendocrine studies

In conjunction with the symposium in Crieff, Scotland 8 years ago, I had the great privilege of visiting with Gerald Lincoln and seeing his highly seasonal Soay sheep. The work since that time (Lincoln & Hazlerigg) has been very productive and led to a concept that annual transitions are generated by birth and death of cells and tissue regeneration throughout the life cycle, which Lincoln & Hazlerigg referred to as “the histogenesis hypothesis”. In Soay sheep, these annual transitions in response to photoperiod occur in tissues of the pars tuberalis of the anterior pituitary gland and in the sub-ventricular zone of the mediobasal hypothalamus. Although they illustrated annual changes regulated by the pars tuberalis with prolactin, they pointed out that in the past, too much emphasis was placed on products without realizing the changes occurring in sources of those products.

Clarke & Smith described the roles and regulation of two relatively recently discovered products, kisspeptin and gonadotropin inhibitory hormone and showed associated histological changes. In 2004, when Bob Goodman and I were preparing the revised chapter on the estrous cycle of the ewe for the Knobil and Neill book (Goodman & Inskeep, 2006), we resisted the suggestion by Associate Editor Tony Plant, that perhaps we should include some mention of kisspeptin, arguing that it had hardly been examined in the sheep. How wrong we were to ignore it! That became clear as Clarke & Smith described how kisspeptin functions in two different hypothalamic areas to regulate GnRH secretion. Particularly striking is the fact that a single cell type contains kisspeptin, neurokinin B and dynorphin, thus mediating both positive and negative feedback of sex steroids. Gonadotropin inhibitory hormone has direct inhibitory effects on GnRH neurons and changes seasonally, both in number of cells producing it (fitting with the histogenesis hypothesis) and in number of GnRH cells contacted by terminals of GnIH cells. The authors noted that seasonal changes require 6 to 9 weeks. Yet seasonal anoestrus can be abrogated by introduction of the male (as discussed in earlier symposia in this series, Walkden-Brown *et al.* 1999). Genetic selection for fertility in May (northern hemisphere) in response to only the male, led to some animals cycling throughout the seasonal period of expected anoestrus (Vincent *et al.* 2000). The mechanism of action of male introduction represents an opportunity for further neuroendocrine studies.

### Sperm quality, storage and function

Sutovsky & Lovercamp reviewed new approaches using biomarkers to assess sperm quality and characterization of the proteome of sperm. Prediction of fertility from examination of semen has long been elusive. A combination of techniques was used to identify ubiquitin, 15-lipoxygenase, thioredoxin SPTRX3/TXNDC8, platelet activating factor-receptor, arylsulfatase A, glycan-specific lectins and acrosome-binding lectins as potentially valuable markers of sperm defects. These molecules show promise for greater accuracy in assessing semen samples using flow cytometry and imaging methodologies.

Sperm storage and movement in the oviduct were discussed by Hung & Suarez, who used a comparative approach and noted that most ruminant work had been done in *Bos taurus*. They identified the uterotubal junction as a key site regulating passage of sperm into the oviduct and discussed how genetic defects, expressed in the proteome of sperm, can prevent that passage. In mice, microscopic observation of the behavior of sperm in the oviduct was a useful technique. Prolonged motile life of sperm is facilitated by binding to cilia of the oviductal epithelium and by delayed capacitation. Capacitation and hyperactivation allow sperm to be released from the oviductal epithelium and move toward the site of fertilization in the ampulla. Each of these processes and whether sperm are guided toward eggs by chemotaxis are not fully understood.

Gadella provided a very extensive review of the fertilization process, including attachment of sperm to the zona pellucida, hypermotility, zona drilling, blocks to polyspermy and sperm-oocyte fusion. His paper was one of the most thorough in the entire conference in citation of the pertinent literature and in analyzing dogma versus knowledge. To one who recalls when Dan Szollosi & Hans Ris (1961) first described the process of fusion of sperm and oocyte membranes to engulf the sperm into the ooplasm in the rat, it seems that knowledge has come a very long way. Gadella, too, provided a new "omics" term, suggesting the need for studies of "glycomics" to further understand the fertilization process.

### Follicles and oocytes

Studies of follicular development and selection have led to interesting progress in understanding that process during the last two decades. One concept detailed here (Smith *et al.*) is that selection of a single ovulatory follicle in the cow may be predicated upon negative regulation of the action of FSH and IGF1 on granulosa cells in follicles destined to become subordinate and atretic. This step occurs after the negative regulatory factor has been down regulated in the lead (dominant, selected) follicle by IGF1 and FSH. The concept arose from characterization of the transcriptome of bovine oocytes. The presence of five expressed sequence tags of mRNA for a neuropeptide, cocaine and amphetamine-regulated transcript (CARTPT), known to have pleiotropic effects, led the Michigan State group to explore its role in the ovary, and ultimately to this concept.

In their paper on the extracellular matrix in the ovary, Rodgers & Irving-Rodgers discussed how focal intra-epithelial matrix (focimatrix), a novel type of basal lamina, may be involved in maturation of granulosa cells. Focimatrix appears before selection of the dominant follicle and increases thereafter. It will be interesting to see if these changes are related to the destruction of CARTPT in the dominant follicle. These authors identified three other roles of extracellular matrix and described variation in its composition in the ovary. They made a case for the bovine ovary as a model for human polycystic ovarian syndrome (known as PCOS), because of its content of fibrillins and their binding to TGF $\beta$  binding proteins. They identified a relationship of oocyte quality to ultrastructure of the follicular basal lamina. And finally, they provided evidence that hyaluronan and versican, matrices produced by granulosa cells, contributed to increases in follicular fluid due to their osmotic potential.

Just as Smith's group has patiently dissected the role of CART in follicular selection in the cow, Murdoch and his colleagues have step-by-step tested components of the mechanism of ovulation in the ewe. They started earlier and with less sophisticated genetic data, but have achieved a similar end. Many of the steps in the ovulatory process fit with the hypotheses in Espey's classic review (1980, updated in 1994). However, Murdoch's work has delineated in the ewe the important role of the ovarian surface epithelium. From that knowledge, they have developed hypotheses for a mechanism by which ovulatory damage to the ovarian surface epithelium can lead to ovarian cancer.

Evans *et al.* have identified greater pregnancy success in animals with greater numbers of antral follicles in the ovary, a trait that is repeatable from cycle to cycle within an animal, and is associated with variations in hormonal concentrations. However, early data indicate that number of antral follicles may be subject to prenatal programming and reduced dramatically by nutritional restriction in the dam during gestation. While ovulation rate in sheep is associated with specific genes, a specific genetic relationship for antral follicle count in cattle appears to be indicated only by breed differences at this time. Daughters of restricted animals did have increased blood pressure and circumference of the aorta at slaughter (a surprising combination) in addition to the effect on follicle number.

### Corpus Luteum

Miyamoto *et al.* found over 4200 papers on corpora lutea in domestic ruminants. They approached luteal development and function as a continuum from formation through gaining function to luteolysis. They especially assessed potential luteotropic roles of growth factors (VEGF, FGF2 and IGFs) and studies of differing sensitivity to  $\text{PGF}_{2\alpha}$  of the early versus mid-cycle corpus luteum. They embraced the concept that life of the corpus luteum depends on the balance of luteotropic and luteolytic factors and hypothesized that  $\text{PGF}_{2\alpha'}$ , well known as a luteolysin, acts as a luteotropin in the early corpus luteum. That postulate is in contrast to the work by Sayre *et al.* (2000) in which repeated injection of  $\text{PGF}_{2\alpha}$  at 8-h intervals up-regulated luteolysis early in the estrous cycle, and it must be evaluated in relation to other data. Consider the early demonstrations by Milvae *et al.* (1986) that other arachidonic acid derivatives have luteotropic or steroidogenic effects early in the cycle. Consider also the enzymatic complement of the corpus luteum as delineated in numerous papers by Michel Fortier, Joe Arosh and their colleagues (e.g., Arosh *et al.* 2004a,b). The enzymatic complement of the ovary includes 9-keto-reductase, which interconverts  $\text{PGF}_{2\alpha}$  and  $\text{PGE}_2$ , the direction depending upon substrate concentration. Numerous studies have established luteotropic effects of PGEs on maintenance of corpora lutea in cattle and sheep (see Weems *et al.* 2006, for review).

The review by Skarzynski & Okuda provided a concept and model of luteolysis in the cow that differs somewhat from the models proposed by McCracken *et al.* (1999), by Niswender *et al.* (2007) in the sheep, by Ginther *et al.* (2010) for natural luteolysis in the cow, or by Weems *et al.* (2006) in their comprehensive review. The current reviewers placed greater emphasis on luteal blood flow and resultant hypoxia. However, they agree that luteal oxytocin is not the only regulator of uterine secretion of  $\text{PGF}_{2\alpha}$  in the cow and is probably not required for that secretion (see Inskeep, 2004 or 2010 for a thorough discussion of timing of luteolysis by progesterone).

Many of the ideas in these papers on corpora lutea must be re-evaluated in relation to the differences in changes during natural (or simulated natural) luteolysis and changes in response to bolus injections of  $\text{PGF}_{2\alpha'}$  as recently documented in a series of papers by Ginther and his colleagues (e.g., 2010).

Hansen and coworkers thoroughly detailed the changes in dogma necessary to accommodate the important new findings (1) that interferon tau is secreted into the uterine vein and that (2) interferon tau has actions in the corpus luteum to support its maintenance during maternal recognition of pregnancy in sheep. This major change needs to be translated rapidly and effectively into the reproductive physiology classroom and textbooks. The linkage of these effects of interferon tau to the anti-luteolytic effects of the E prostaglandins deserves further examination and clarification in future research (Pratt *et al.* 1977, 1979; Silvia *et al.* 1984; Weems *et al.* 2006; Lee *et al.* 2010; McCracken *et al.* 2010; Stephen *et al.* 2010). Hansen *et al.* also discussed the induction of interferon stimulated glycoproteins in blood cells, which parallels secretion of interferon tau and provides a diagnostic test for pregnancy.

The camelid placenta does not produce progesterone, so all camelid species are dependent on the corpus luteum throughout pregnancy. In dromedary camels, Skidmore *et al.* found that progesterone-treated recipients that had not ovulated could get pregnant from transferred embryos. To avoid continued daily injections, they induced new corpora lutea later by treatment with eCG and GnRH. Interestingly, 50% remained pregnant after withdrawal of exogenous progesterone, a proportion equal to that observed in cattle in which a new corpus luteum was induced during days 28 to 36 of pregnancy (Bridges *et al.* 2000), after previous maintenance by exogenous progestogen during absence of a corpus luteum.

### The lactating dairy cow

Despite the variety of assigned subjects, 7 of the speakers centered some or all of their concerns on the lowered fertility rates of the high producing, lactating dairy cow (Santos *et al.*, Evans *et al.*, Lonergan, Sartori *et al.*, Burke & Verkerk, Wiltbank *et al.*, and Smith *et al.*) and an 8<sup>th</sup> (Zicarelli) addressed similar problems in dairy buffalo, a species that is gaining in numbers more rapidly than cattle. Numerous factors have been identified as contributing to success or failure of pregnancy in these animals. These factors include concentrations of estradiol or progesterone at critical periods, antral follicle counts in the ovaries, temperature stress, concentrations of non-esterified fatty acids during negative energy balance in the early postpartum period, timing of hormonal treatments and management factors.

As an example of the factors listed above, low progesterone during (1) the cycle before breeding, (2) the first few days after breeding, (3) maternal recognition of pregnancy, or (4) placentation, has major negative impacts on pregnancy success (Inskeep & Dailey, 2005). However, various attempts to overcome low progesterone at these times have met with limited or sporadic success.

Similarly, inadequate exposure to estradiol during the immediate preovulatory period can interfere with oocyte maturation and luteal function or lifespan. Wiltbank *et al.* discussed the metabolism of steroids in high producing cows and Day *et al.*, from work in beef cattle, pointed out that treatment with estradiol in regimens for synchronization of oestrus can be beneficial. In cows in which duration of proestrus was shortened by treatment with GnRH, life span of the corpus luteum in beef cows was less than 12 days in 74% of 38 animals in which duration of proestrus averaged only 1.3 days before induced ovulation, compared with only 30% of 40 animals in which duration of proestrus averaged 2.3 days (Mussard *et al.* 2007; Bridges *et al.* 2010). Cordoba & Fricke (2002) found short cycles in 51% of 49 lactating dairy cows that returned to service after timed insemination on an OvSynch program.

Prevention of early secretion of PGF<sub>2α</sub> (which causes a short luteal phase; reviewed by Inskeep, 2004 and Inskeep & Dailey, 2005) in response to the post-ovulatory rise in progesterone required a sequence of pre-ovulatory exposure to progesterone and estrogen (Kieborz-Loos *et al.* 2003). That sequence has not occurred at the first ovulation at puberty or after parturition, and can be lacking if follicles are ovulated prematurely in other situations. Thus, if follicular maturation, and hence exposure of the reproductive tract to estrogen, was inadequate, premature secretion of PGF<sub>2α</sub> could be the reason for occurrence of short luteal phases after GnRH. Conversely, excessive estradiol can damage the oocyte (as in the case of persistent follicles; Cooperative Regional Research Project NE 161, 1996), interfere with maternal recognition of pregnancy (Breuel *et al.* 1993), or disrupt placentation (Bridges *et al.* 2000).

Particularly valuable in understanding limitations to fertility in the early postpartum period has been the innovative work by Leroy *et al.* (2008a,b), discussed in the review by Lonergan.

They showed that increased non-esterified fatty acids in follicular fluid during negative energy balance compromised oocyte and embryo quality.

#### *Are there solutions for management of the lactating dairy cow?*

A particularly challenging dilemma is the continued decrease in reproductive performance of high-yielding, lactating dairy cows. There is a clear need for further research into the basic mechanisms of reproductive performance in the dairy cow, as discussed thoroughly by Lonergan. He pointed to follicular development, poor exhibition of oestrus, lowered oocyte quality, altered sperm transport, suboptimal reproductive tract environment and embryonic and fetal losses as contributors and the need to determine the relative contributions of each to lowered fertility rates within a given situation.

There is an eminent need for innovative thinking about the utilization of what is already known. The insistence that breeding in large herds must be tied to timed ovulation, without estrous detection, has been shown to be helpful in pregnancy rate in some herds (Wiltbank *et al.*, Santos *et al.*). In others, it may contribute to the problem, rather than the solution. Particularly, it may promote selection against expression of oestrus. Treatments to control variables and avoid heat detection have become ever more complicated as illustrated by Wiltbank *et al.* for the dairy cow and Day *et al.* for the beef cow. Variations in these methods have been necessary in Zebu cattle (Sartori *et al.*) and water buffaloes (Zicarelli). Water buffaloes are especially compromised by seasonality and by the nearly complete lack of homosexual behavior in the female (Usmani *et al.* 1983). Like the dairy cow, buffaloes are encumbered by high late embryonic mortality. Sartori *et al.* noted the smaller antral follicles and corpora lutea in *Bos indicus* compared with *Bos taurus* and the lowered dosages of hormones needed because of greater feedback sensitivity. Burke & Verkerk discussed how the increased constraints of both larger herd size and external influences of consumers, environmental issues and animal welfare concerns affect the more extensive production system in New Zealand. New Zealand dairy- ing is seasonal and supports the largest sector of agricultural exports. They reviewed practices developed, including effectiveness, shortcomings and side effects, and supported a need for simpler schemes of treatment for reproductive management.

R. A. Dailey has proposed a simpler scheme of synchronization that would not use timed breeding, but require observation for oestrus just 3 days in each three-week period (presented in detail in Inskeep & Dailey 2010). However, when that scheme was submitted to use in two herds, analyses of the data collected revealed only 54% complete compliance with even that simple scheme (RA Dailey, unpublished); non-compliant inseminations have been reported as a problem with more involved schemes (Stevenson & Phatak 2005).

Rodríguez-Martínez *et al.* (2008) compiled data showing that duration of oestrus had changed over time, especially in American Holsteins. Although Rottensten & Touchberry (1957) reported a heritability of 21%, duration of oestrus has not been considered in selection programs. Exhibition of a detectable oestrus allows proper timing of insemination, and is indicative of appropriate maturation of the oocyte and readiness of the reproductive tract to establish pregnancy. Sartori *et al.* noted that when groups of synchronized Holstein and Nelore cows were run together, approximately 90% of mounts were within breed.

In early studies (Inskeep *et al.* 1961), conception was defined as birth of a live calf and inheritance of conception rate at first service was examined in Holsteins bred to high fertility AI sires (60- to 90-day non-return rate in the upper two-thirds of the bulls in the stud). Heritability of conception rate to first service, estimated from intra-sire correlations of paternal half-sib groups, was approximately 8.5%. Based upon that estimate, if one were to select daughters of bulls in

the upper 25% of sires for daughter's conception rate, based upon 20 previous daughters, the expected advantage would be 4.7% in the next generation. Selection based upon conception rate to first service has not been implemented, but some schemes now utilize daughter pregnancy rate. More recently, Bamber *et al.* (2009) estimated the heritability of pregnancy loss after 30 days as 49%, which should make that trait a more effective tool for selection.

In a different approach, Khatib *et al.* (2008) found that the gene, signal transducer and activator of transcription 5A (*STAT5A*), was associated with sperm factors that caused low fertilization rate *in vitro*, with death rates of *in vitro* fertilized embryos, and with milk composition in Holstein cows. It appeared that selection for heterozygotic females could promote both successful pregnancy and increased production.

Loneragan illustrated changing attitudes in selection programs to put less emphasis on production and more emphasis on reproduction and health. Selection for strength and duration of expression of oestrus and for conception to first AI, against pregnancy loss, or for specific genes that regulate components of the reproductive process seem appropriate to recommend. These approaches may prove far more valuable to sustainability of dairy production than the extensive efforts at minor modifications in hormonal treatments designed to facilitate timed breeding. Santos *et al.* illustrated successes on farms that have adopted integrated approaches, including consideration of cow comfort, transition cow management, aggressive postpartum health monitoring and manipulation of the ovarian cycle to increase insemination rate, as well as introduction of selection for fertility.

### Other new findings

This reviewer was surprised to learn that the family Camelidae originated in North America (Skidmore *et al.*); they seem more plentiful on other continents. Progress in facilitating AI and embryo transfer was achieved in dromedary camels by synchronizing follicular waves using GnRH and prostaglandin analogues.

Preservation of endangered wild species is an increasing concern in studies in zoos throughout the world and has received increasing emphasis. Just last week, expectation of birth of a Sumatran rhinoceros from AI was announced at the Cincinnati Zoo. Santiago-Moreno *et al.* shared their experience in sperm collection and preservation in the Spanish ibex. Males with larger, more symmetrical horns had better semen quality, thus aiding selection of candidate males. They have developed a captive breeding program, synchronized ovulation using a method that was effective in dairy goats and obtained fertility rates of 25 to 63%. Interestingly, only dominant females became pregnant. In addition, they have produced live hybrids with domestic goats. As in other wild ruminants in the Mediterranean region, seasonal reproductive activity began earlier in males than in females.

In Alaska, reindeer and caribou, members of the same species, are of great interest. Reindeer are the only cervids indigenous to the arctic environment (Shipka & Rowell) and the only deer in which male and female both grow antlers. The females do not show homosexual behaviour. Their gestation length has been the subject of great variation in fragmentary reports. The data showed that they are seasonal breeders with an estrous cycle of 18 to 29 days, mean 24 days. Gestation length was quite variable, from 198 to 229 days and depended on when in the season the female was bred. The variability makes them somewhat comparable to the horse, but the effect of season on timing makes one wonder what mechanism is operating that contributes to such wide variability. Progesterone in blood rose more rapidly in animals bred later, which could be a clue to that mechanism.

### Final remarks

Drs. Michael Smith and Matthew Lucy asked me to predict where we are headed in studies of reproduction in ruminants; what is not known and needs to be learned. I have interspersed some suggestions derived from the reviews presented by the 31 speakers at the symposium. It is clear that such thoughtful reviews can help to make planning research more efficient, that tomorrow's researchers will need to be more widely read and that more cost effective methodologies will help with the research task. Even so, funding will be a limiting factor. I believe we should be thinking more and publishing less.

New methods for genetic studies will enable more readily identified genes for study. That will provide opportunities for programmatic, sequential studies that lead from discovery of a gene to understanding the role of its product. There will still be a need for basic physiological studies of that product (example CART) and its actions and for application of knowledge in applied research.

Finally, there is a great need for unbiased dialogue among workers who are operating with different models of their understanding of a process or system. *Dogma needs to be challenged!* That is the road to greater understanding and challenging it together should help to fulfill Carl Hartman's statement in the early days of our discipline, "Science should be fun!"

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