Reproduction in domestic ruminants during the past 50 yr: discovery to application

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ABSTRACT: The study of reproductive physiology in domestic ruminants has progressed from the whole animal to the molecular level in an amazingly short period of time. The volume of information on this subject is enormous; therefore, we have focused on domestic ruminants, with an emphasis on cattle. To date, artificial insemination (AI) is perhaps the most powerful technique that reproductive physiologists and geneticists have provided the livestock industry for genetic improvement. Early efforts to establish AI as a tool were initiated in Russia around 1899 and since that time major advances in methods of semen collection, evaluation of male fertility, cryopreservation of sperm, sex-sorted semen, and estrous cycle control have occurred. The preceding advances not only led to the widespread use of AI, but also contributed to our fundamental understanding of ovulation control, timing of insemination, gamete biology, and cryopreservation. In regards to anestrus, our understanding of the concept of neuroendocrine control of the pituitary gland and the role of steroid feedback led to the Gonadostat Theory, which proposes that onset of puberty is due to a decrease in the negative feedback of gonadal steroids over

time. Subsequent studies in prepuberal and postpartum sheep and cattle established that a short luteal phase frequently precedes the first normal length cycle that is accompanied by estrous expression. This observation led to the common practice of treating prepuberal heifers and anestrous postpartum cows with a short-term progestin treatment (e.g., Controlled Internal Drug Release) to induce normal estrous cycles. In domestic ruminants, fertilization rate is high (85% to 95%); however, significant embryonic mortality before or around the time of maternal recognition of pregnancy (MRP) reduces the pregnancy rate to a single breeding. Significant effort has been directed at determining the time of MRP, the signal for MRP, as well as elucidating the physiological, cellular, and molecular dialogue between the conceptus and uterine environment. Advancements have now led us to the ability to edit the genome to alleviate disease and possibly improve production traits. In summary, major advancements in our understanding of reproductive biology have stemmed from efforts to establish the AI and embryo transfer technique and reduce the negative impact of anestrus and embryonic mortality in domestic ruminants.

Key words: history, reproductive physiology, ruminants

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INTRODUCTION

Reproductive physiology has a long and distinguished history, beginning with the detailed anatomical study of male and female reproductive

organs in the 1600s, progressing to the use of “ablation and replacement” as a tool for elucidating basic physiological and endocrine mechanisms, and more recently the use of CRISPR Cas9 technology for gene editing (Jinek et al., 2012). Although the first written observations on ruminant reproduction were likely made by Aristotle around 300 BC (Short, 1977), the period from 1666 to 1676 has been designated a decade of

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discovery based on the following observations: 1) it was proposed that ovaries contain eggs, 2) Regnier De Graff hypothesized that ovarian follicles contain eggs, 3) the corpus luteum (CL) was named, 3) De Graff reported that corpora were transient, provided an estimation of the number of embryos in the uterus, and disappeared following parturition, and 4) spermatozoa were first visualized by Hamm and Leeuwenhoek (reviewed by [Cobb, 2006](#)).

The underlying rationale for reviewing some of the major scientific discoveries in ruminant reproduction was probably best described by Keith Betteridge, a distinguished Canadian reproductive biologist, who wrote that “The vitality of a discipline benefits from a knowledge of its roots.” ([Betteridge, 2000](#)). Because the volume of information on this subject is enormous, we have chosen to focus the paper on some of the seminal discoveries in domestic ruminants, with an emphasis on cattle. In particular, we will discuss specific biological processes required for the establishment and maintenance of pregnancy, with an emphasis on findings that resulted in management strategies to increase reproductive efficiency or genetic merit. For excellent reviews on advances in female reproductive physiology in dairy cattle over the last 100 yr, see [Moore and Hasler \(2017\)](#), [Stevenson and Britt \(2017\)](#), and [Thatcher \(2017\)](#).

DEVELOPMENT OF ARTIFICIAL INSEMINATION

In this section, we focus on artificial Insemination (AI) as it relates to semen collection, processing, and evaluation with an emphasis on the bull. The modern history of AI begins in 1899 in Russia when Professor Ivanov (translated also as Ivanow and Ivanoff) used sponges to recover semen from the vagina of mares following mating (see [Ivanoff, 1922](#)). Within a decade, his laboratory was evaluating semen collected from domestic farm animals including horses, cattle, sheep, rabbits, poultry as well as the dog and fox ([Ivanow, 1907](#)). Soon bulls were being ejaculated via rectal massage of the accessory sex glands with semen collected in funnels and tubes. A major advance was made when the first artificial vagina was developed for bulls in 1914. Yet bulls continued to be collected via rectal massage until as late as 1920. The advantage of the artificial vagina was that bulls could now be collected off of females or even other males without contamination from the female or need for rectal palpation. Similar artificial vaginas were also developed for sheep at the same time.

Publication of Ivanov’s results in 1922 resulted in a flurry of research on semen in Europe, Japan, and the United States. The AI of cows in Russia reached 19,800 in 1931 (see [Walton, 1933](#)). Success of AI in dairy herds was reported in New York State, Minnesota, Missouri, and Wisconsin in the early 1930s (see [Foote, 2005](#)). The dairy cattle industry understood the potential advantage for the use of AI and the first AI cooperative was established in Denmark in 1936 with cooperatives in New Jersey and New York State following in 1938. It was quickly recognized that the success of AI in cattle needed improvement. There was a need to develop better techniques for bull management, semen collection, semen preservation, and insemination protocols. This is where the focus shifted during the 1940s, 1950s, and 1960s.

Semen for AI prior to the 1960s was mainly used as liquid semen. The semen was diluted with a medium called an extender to preserve the sperm’s life. The initial work utilized physiological saline that may have included a sugar such as fructose or glucose (see [Salisbury et al., 1978](#)). In 1940, Phillips and Lardy developed the phosphate buffered egg yolk solution that consisted of a phosphate buffered saline diluted with egg yolk. Phillips believed that a readily available natural substance could sustain sperm and was the basis for selection of egg products. Egg whites alone did not work but buffered egg yolk did. In 1941, Salisbury et al. discovered that citrate could replace the phosphate buffer in an egg yolk extender (EYC) and had the added effect of making the extender relatively clear. The phosphate egg yolk extender had large fat globules present that obscured sperm from view in a microscope. In 1945, the EYC extender was modified to include glucose ([Salisbury and VanDemark, 1945](#)). From 1940 to 1952 semen was transported at room temperature from AI cooperatives/Bull studs via the mail, bus, train, and air plane drop. Samples of semen included multiple breeding doses in a tube. A key was the arrival of semen within 24 h from collection, as that was the extent of semen fertility under these conditions. In 1949, Foote and Bratton reported that egg yolk and milk lipoproteins could protect sperm against the damages of cooling to 5 °C, cold shock, as long as semen was first pre-extended. Further at 5 °C, sperm could survive for up to 3 d with a 7% improvement in fertility. Shortly after, several authors reported sulfanilamides or antibiotics could decrease bacterial count in bovine semen and increase fertility another 5% ([Almquist et al., 1949](#); [Foote and Bratton, 1950](#)). Both cooling and antibiotics were quickly adopted by the AI

industry enabling farther shipping of semen and in single dose units.

Further extender modification during the 1950s included the development of heated milk extenders (O'Dell and Almquist, 1957) and the observation that egg yolk, but not milk, inactivates some antibiotics (Elliott et al., 1962) so antibiotics should first be added to neat semen. In the 1960s, an effort was made to improve liquid semen extenders. The Cornell University Extender (CUE; Foote et al., 1960) and Illinois Variable Temperature Extender (IVT; Bartlett and VanDemark, 1962) were developed to maintain pH and either generate CO₂ via bicarbonate-citric acid buffer systems or be gassed with CO₂. These extenders in effect kept the concentration of oxygen down during sperm storage, minimizing reactive oxygen species formation. The CUE extender was used as the base for the later development of Caprogen (Shannon, 1965) which included catalase as an antioxidant (see Shannon et al., 1984). Caprogen was also shown to give excellent fertility when semen was extended and held at ambient temperature for 1 d. The tris-egg yolk extender (EYT) was first developed in 1963 (Davis et al., 1963). Peak semen fertility was reached at this point with cooled liquid semen developed to maximize sperm survival and inhibit bacterial growth.

The change to use of frozen semen first required the development of the technology and extenders to preserve sperm viability. In 1949, Polge et al. first reported the cryopreservation of sperm using glycerol and quickly demonstrated bull sperm could also be cryopreserved (Smith and Polge, 1950). Serendipity was critical in this discovery (Polge, 1968). Polge was initially testing if sugars would dehydrate sperm and protect them during freezing. While he was not successful, he tried repeating the experiments 6 mo later. One of the stock solutions proved successful but the label had fallen off. Chemical analysis revealed it was Meyer's albumin that contained glycerol. It was the glycerol that was the key to success. Soon it was discovered that cryosurvival of sperm was best if glycerol was added after sperm were cooled to 5 °C (Polge, 1953). This culminated in the first calves from frozen semen in 1952 (Polge and Rowson, 1952). Initial studies of cryopreservation used semen frozen in a dry ice alcohol slurry. Cold shock was discovered when semen was cooled too quickly to 5 °C prior to freezing and it was observed that egg yolk or milk in extenders could prevent these effects (Blackshaw, 1954). Fortunately, the extenders containing egg yolk, EYC and EYT, and milk proved very useful for cryopreservation when glycerol was added

(see Foote, 2005). By 1957 several critical features of freezing extenders were developed that have not changed since. The optimal level of glycerol was established as 7% for both egg yolk and milk based extenders (Blackshaw et al., 1957) and the optimal level of egg yolk was found to be 20% (VanDemark et al., 1957; see Salisbury et al., 1978).

It was also discovered that cryostorage of sperm was best at -79 °C as compared to warmer temperatures (Davis et al., 1963). This is the temperature of dry ice and was difficult to maintain a storage system. Liquid nitrogen was looked at as an alternative that maintained colder temperatures of -196 °C, but systems for routine storage were not developed until the late 1950s. These were developed by American Breeders Service and quickly adopted throughout the AI industry (see Foote, 2002). During the 1960s, modifications of extenders included the addition of antibiotics to neat semen to improve fertility, as egg yolk can inactivate antibiotics (Elliott et al., 1962). Egg yolk or milk was needed in the extender for cooling to 5 °C and also while freezing over liquid nitrogen for optimal survival (see Foote, 2002). The protective role of egg yolk and milk against cold shock was due to lipoproteins, phospholipids, lecithin, and(or) casein (Salisbury et al., 1978). Storage or equilibration of semen at 5 °C for 4 h prior to freezing was found to improve cryosurvival (Benson et al., 1968). Up until the end of the 1960s semen was thawed in ice water. It was discovered that warm water thawing was more advantageous, but had practical limitations as no device was yet available to stabilize water temperatures. In the 1970s, semen was demonstrated to survive storage in liquid nitrogen for years but storage was better at a central location, for example, at the AI organizations as opposed to on farm storage (see Foote, 2002). The factors on why storage on the farm were a problem were identified, but often related to lack of appreciation of the delicate nature of the liquid nitrogen tank and the need to keep semen storage canisters covered by liquid nitrogen. Previously identified warm water thaw temperatures were standardized to 35 °C to balance increased sperm survival with ease of use on farm (see Salisbury et al., 1978). It was noted that a shorter thaw at 75 °C did improve post thaw viability but was not practical for routine use (Rodriguez et al., 1975). Interestingly, milk extenders seem to have a much broader range of optimal thawing conditions including thawing in air surrounded by a paper towel. Multiple researchers identified interactions of glycerol level, freezing rate, thawing rate, and extender that led to different conditions for

optimal cryosurvival of sperm with different combinations of these factors (Saacke, 1978).

By 1978, Dr R. G. Saacke noted "There is no indication that the future will be different from the past. New innovations will come along and much of semen processing will again be open to re-evaluation. Basically, we are talking about what researchers refer to as interactions."

Semen packaging systems have evolved with the previously noted changes in semen extenders and distribution systems. From 1900 to 1957, semen was shipped as liquid semen in capped glass and later plastic tubes. While a version of the straw was developed in Denmark early on (Sørensen, 1940) it was not adopted until much later. Rather tubes were replaced by sealed glass ampules in the 1950 for liquid semen and later adapted for freezing with dry ice alcohol baths. As liquid nitrogen technology evolved, a new storage and delivery system was needed and the 0.5 mL French straw was developed by Cassou (1964). Mechanical means for sealing straws and guns for insemination were developed in 1974 by Pickett and Berndston. The French straw has since been modified to be either 0.25 or 0.5 mL with the same total number of sperm used in either type of straw. During the development of the straw an alternative system with semen frozen on dry ice as pellets was developed (see Salisbury, 1978). While simple, it was not easy to label individual doses.

Semen collection procedures have undergone considerable improvement since bulls were initially collected via rectal massage of accessory sex glands (Ivanow, 1907). The initial artificial vaginas developed for bulls in 1914 (see Walton, 1933) remained in use without much change until cold shock was found to be detrimental to semen quality. Salisbury demonstrated the need to keep the artificial vagina warm during collection and semen warm through initial evaluation and dilution of the semen with an extender (Salisbury and Willet, 1940). A series of studies in the 1950s through 1970 demonstrated that the amount of semen collected could be increased by use of false mounts, moving the bull or teaser back and forth, introducing a new teaser, or moving the bull and teaser to a new location (Almquist, 1973; Foote, 2002). It was also found that increasing the number of times a bulls was collected from 1 to 2 times per wk increased sperm collected but without a decline in fertility (Bratton and Foote, 1954; Hafs et al., 1959). The electroejaculator was developed by Dziuk et al. in 1954 but was used primarily for beef bulls. Beef bulls were not collected frequently enough to sufficiently train for semen collection with an artificial vagina. In 1963, Foote and Heath

recognized that a shorter artificial vagina yielded more sperm and has since been adopted. In 1966, MacMillian et al. recognized the importance of a warm artificial vagina above body temperature and the need for sufficient pressure between the liner and outer casing. Many of the factors described above to maximize semen collection were not so important when mature 5-yr-old bulls were collected by the AI organization. At that time, mature bulls were collected due to the time needed to acquire progeny proofs to predict the genetic transmitting ability of a bull (Henderson, 1954). This changed with the development of genomic proofs resulting in younger bulls comprising the bulk of the bulls in the studs (VanRaden et al., 2009; Aguilar et al., 2010). In 2016, most bulls are less than 2 yr of age and hence lower daily sperm production and maximizing collection efficiency is critical (Parrish, 2016). Bulls are often collected as soon as puberty is achieved, a time during which insufficient sperm are produced for routine use in AI. That semen may be used for in vitro fertilization instead.

Evaluation of which semen is useful for AI has undergone changes over time. The basic criteria for normal sperm was established in 1947 by Mercier and Salisbury, sperm viability assays by histochemistry in 1950 by Blom, and microscopic evaluation of sperm motility by Bratton et al. in 1956. A more recent summary of bovine sperm morphology is described in Barth and Oko (1989). Other critical observations pertaining to sperm quality and evaluation included the following: 1) motility was shown to be temperature dependent, 2) microscopic techniques influenced ability to visualize sperm, 3) the type of extender and sperm concentration proved to be important, and 4) the time of semen evaluation, whether immediate or after storage was critical (see Foote, 2005). Early motion analysis with still images or film by Rothschild (1953) and Rikmenspoel et al. (1960) eventually led to the development of computer added sperm analysis (CASA) currently in use. Other advances in evaluating sperm included the quantitation of acrosomal morphology (Saacke and Marshall, 1968), in vitro fertilization (Parrish et al., 1986), fluorometric assays of mitochondrial function and sperm viability (Garner et al., 1997), and flow cytometry evaluation for sperm DNA, mitochondria, acrosome, and viability (Graham et al., 1990). Early on it was recognized a combination of tests to evaluate semen would predict semen fertility (Bratton et al., 1956) and this has been born out in numerous studies since that initial report (see Salisbury et al., 1978; Foote, 2002).

A major recent change to AI has been the introduction of sex-sorted semen (Johnson and Seidel, 1999; Garner and Seidel, 2008). The technology is based on the amount of DNA in the X and Y chromosome being different (Gledhill, 1985). Flow cytometers that sort cells have been adapted to isolate X- or Y-bearing sperm. Speed and improvements in quality of sperm post sort and then following cryopreservation have made this a routine method to improve the percentage of females obtained. While in theory it also works to produce males, this does not have the same economic benefit as production of females, particularly in the dairy industry where this is most often used. Currently, the fertility of sex-sorted semen is between 5% and 10% less than unsorted semen from the same bull (Vishwanath, 2014), but this is expected to narrow with improvements in procedures and equipment.

More detail on aspects of semen collection, processing, and evaluation in the bull can be found in several books and reviews (Emmens and Blackshaw, 1956; Perry, 1968; Salisbury et al., 1978; Cupps, 1991; Foote, 2002; Foote, 2005). There is additional work in the ram that could not be discussed due to space (Salmon and Maxwell, 1995, 2000).

ESTROUS CYCLE AND OVARIAN FUNCTION

While major advancements were being made in methods of semen collection, extension, and preservation, between the 1920s and 1940s, there was a parallel research effort focused on female ruminants that facilitated the commercialization of AI. Although a general knowledge of duration of estrus and estrous cycle length in cattle was known, detailed information was not available until the early 1900s (reviewed by Hammond, 1927). One of the early pioneers of reproductive physiology in the United States was Fred McKenzie, at the University of Missouri, who not only investigated methods of semen collection and preservation in farm animals, but initiated a series of studies on the estrous cycle of ewes, sows, and mares. McKenzie's research philosophy emphasized the importance of elucidating basic physiological mechanisms before attempting to manipulate reproductive events. Establishment of the metrics of the estrous cycle and time of ovulation relative to estrus expression in cattle helped determine the optimum time for AI. Utilizing breeding data, visual observation of estrous behavior, and palpation per rectum, Trimberger and Davis (1943) established the AM/PM rule in which cows

detected in estrus in the morning are inseminated in the evening and vice versa. This recommendation is still widely used today.

Hormonal Control of the Estrous Cycle

As early as 1905, Walter Heape reported that ovulation is induced by mating in rabbits, providing evidence for the neuroendocrine control of ovarian function. The link between the pituitary gland and ovary was firmly established when P. E. Smith (1932) showed that hypophysectomy inhibited ovarian activity and that implanting pituitary tissue restored ovarian follicular growth in rats. Initially the pituitary gland was considered to be the "master gland" of the endocrine system; however, the concept of neuroendocrine control of pituitary function emerged during the 1930s and 1940s. Following the discovery of the hypothalmo-hypophyseal portal vessels (Popa and Fielding, 1930), Geoffrey Harris (Cambridge, United Kingdom) conducted a series of experiments that provided compelling evidence for the hypothalamic control of pituitary function (Harris, 1955). The concept of hypothalamic neurosecretion leads to an intensive effort to identify specific hypothalamic releasing (e.g., gonadotropin releasing hormone [GnRH]) and inhibiting factors (e.g., Somatostatin; Wade, 1981). More recently, the role of kisspeptin in controlling GnRH secretion in sheep (Smith et al., 2014) and cattle (Amstalden and Williams, 2014) has been investigated. Reproductive management of cattle has benefitted from the commercial availability of GnRH and analogs of GnRH, which have been used to synchronize follicular waves, induce ovulation, and treat cystic follicles.

The purification of estradiol (1923), progesterone (1934), testosterone (1935), LH (1940), and FSH (1949) occurred between 1920 and 1949, and this period has been described as the "Heroic Age of Reproductive Endocrinology" (Medvei, 1982). Hormone concentrations were initially measured by bioassays (e.g., ovarian ascorbic acid depletion assay or the uterine weight bioassay). Although these assays were useful for hormone purification and generating a preliminary understanding of the estrous cycle, development of the radioimmunoassay (RIA; Yalow and Berson, 1959) transformed the field of endocrinology. Rees Midgely and Gordon Niswender made an enormous contribution to ruminant reproduction by developing RIAs for reproductive hormones, including ovine and bovine LH (Niswender et al., 1969) and this publication was identified as a "citation classic."

Radioimmunoassays are more sensitive, specific, and precise than bioassays and allow large numbers of blood samples to be analyzed in a single assay.

Bill Hansel, Cornell University, was an early adopter of this technology and utilized RIAs to characterize circulating concentrations of LH, estradiol, and progesterone during the estrous cycle of the cow, sheep, and pig (Hansel and Echterkamp, 1972). In the preceding paper, they reported the following findings, which have proved to be essential to our understanding of the estrous cycle in ruminants: 1) there is an inverse relationship between circulating concentrations of LH and progesterone, 2) LH has a role in maintaining luteal function, 3) the preovulatory increase in estradiol is preceded by luteolysis, 4) the preovulatory increase in estradiol appears to induce the preovulatory surge of LH, and 5) luteolysis is not due to a decrease in circulating concentrations of LH, but a uterine luteolytic factor. Since the preceding publication, endocrine data generated by the RIA technique have been instrumental in forming our current understanding of the endocrine patterns of gonadotropins, ovarian steroids, and prostaglandins during the prepuberal period, estrous cycle, pregnancy, and postpartum interval of domestic ruminants.

Advancements in Our Understanding of Ovarian Function

A comprehensive review of the major advancements in our understanding of ovarian function in ruminants, including follicular formation (Juengel and Smith, 2014), primordial follicle activation (Fortune et al., 2013), identification of intrafollicular regulators of follicular development (Juengel et al., 2013; Campbell et al., 2014), development of the two cell–two gonadotropin concept for estradiol production (Fortune and Quirk, 1988), and mechanisms associated with ovulation (Murdoch et al., 2010) is beyond the scope of this paper. Instead, we chose to briefly discuss the characterization and manipulation of follicular waves in cattle.

Several mammalian species have been characterized as having a single (rodents and pigs) or multiple (sheep, cattle, horses) follicular waves during an estrous cycle (Fortune, 1994). In cattle, follicular waves are initiated before puberty and continue during the estrous cycle, most of gestation, and the postpartum anestrous period. In 1960, Rajakoski first proposed that cattle have 2 follicular waves during the estrous cycle. In 1984, the presence of follicular waves, during the bovine estrous cycle was

confirmed by transrectal ultrasonography (Pierson and Ginther, 1984; Reeves et al., 1984). Normally, there are 2 or 3 follicular waves per cycle, beginning with recruitment of a cohort of small antral follicles followed by selection of a dominant follicle that either becomes atretic or ovulates (Sirois and Fortune, 1988). For an excellent historical review of the experiments that lead to an understanding of dominant follicle turnover during the bovine estrous cycle see Ireland et al. (2000). Reviews on the physiological and molecular mechanisms regulating follicular waves have been published elsewhere (Fortune et al., 2001; Lucy, 2007).

In the absence of a CL, low circulating concentrations of progesterone extended dominant follicle life span (Sirois and Fortune, 1990) and decreased pregnancy rates to the subsequent estrus (Hansel et al., 1961; Cooperative Regional Research Project, 1996). Extension of the dominance phase of the ovulatory follicle (“persistent follicle”) provided insight into an earlier observation that pregnancy rates were reduced when heifers or cows were inseminated at the estrus immediately following long-term progestin treatment (e.g., 18 to 20 d; reviewed by Lauderdale, 2009). Consequently, modern estrous synchronization protocols have been designed to minimize the probability of a persistent follicle being present at insemination in cattle. Characterization of follicular waves in cattle resulted in efforts to synchronize waves for the purpose of more precisely controlling the time of ovulation. Synchronization of follicular waves following injection of GnRH (e.g., United States) or estradiol (e.g., Brazil) is a common practice in modern fixed-time AI protocols.

Regulation of CL Function

Significant advancements have been made in our understanding of the development of the CL (Smith et al., 1994), regulation of luteal progesterone synthesis (Niswender et al., 2007), role of the uterus in regulating luteal life span (Ginther, 1976), and the endocrine and molecular mechanisms associated with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)-induced luteolysis in ruminants (Niswender et al., 2000), including the role of the immune system (Walusimbi and Pate, 2013). However, for the purpose of this paper, we chose to briefly discuss the events that lead to the discovery of $PGF_{2\alpha}$ as the uterine luteolysin and the eventual commercial application of $PGF_{2\alpha}$ for purposes of estrous synchronization in cattle.

In 1923, Leo Loeb reported that hysterectomy in the guinea pig extended luteal life span and he hypothesized that “an internal secretion of the uterine mucosa might have a specific abbreviating effect upon the corpus luteum” (Loeb, 1923, 1927). However, the significance of this observation was not pursued for a number of years. Wiltbank and Casida (1956) reported that hysterectomy caused maintenance of luteal function in cattle and subsequently a number of other investigators reported similar findings in other species. In the 1930s, fresh semen was shown to cause uterine contractions (Kurzroc and Lieb, 1930; von Euler, 1935) and this led to the discovery of a class of molecules called prostaglandins, for which in 1982 the Nobel Prize in Physiology or Medicine was awarded to Sune Bergström, Bengt Samuelsson, and John Vane. However, in the 1930s there was no clear evidence that prostaglandins had a role in ovarian function. In 1965, there was a conference entitled “Ovarian Regulatory Mechanisms” which was sponsored by the UpJohn Company. During the discussion of a talk by Bill Hansel (Cornell University) on luteotropic and luteolytic mechanisms in bovine corpora lutea, John Babcock, a chemist working for UpJohn Co.), said “I wonder if anyone here has thought of the possible role of a family of agents known as prostaglandins?It has been speculated that they may play a role in fertility because they are found in very high concentrations in the semen of some species. Whether or not release of prostaglandins from the uterus could have a luteolytic effect, I have no idea.” (Hansel, 1966). In the audience was Bruce Pharriss, who was also an employee of the UpJohn Co. He returned to the lab and initiated a series of experiments to examine the luteolytic action of $\text{PGF}_{2\alpha}$ and reported that injection of $\text{PGF}_{2\alpha}$ into the uterus induced luteolysis in rats (Pharriss and Wyngarden, 1969). Subsequently, John McCracken and coworkers (1972) provided compelling evidence that $\text{PGF}_{2\alpha}$ is the uterine luteolysin in sheep and that same year $\text{PGF}_{2\alpha}$ was reported to induce luteolysis in cattle (Lauderdale, 1972; Liehr et al., 1972; Rowson et al., 1972). Subsequently, Jim Lauderdale (UpJohn Co.) initiated a series of experiments that generated the data which led to FDA approval of $\text{PGF}_{2\alpha}$ for synchronization of estrus in cattle. FDA approval of $\text{PGF}_{2\alpha}$ occurred for horses in 1976, beef cattle and dairy heifers in 1979, and lactating dairy cows in 1983. The development of a $\text{PGF}_{2\alpha}$ analog was reported in 1974 (Cooper, 1974). Importantly, $\text{PGF}_{2\alpha}$ is the most frequently used estrus synchronization product in current

protocols for synchronizing estrus and ovulation in ruminants (Lauderdale, 2009).

The ability to precisely control estrous expression to facilitate the use of AI in cattle has been a long-term goal. Hammond (1927) reported that removal of the CL on day 6 or 7 resulted in estrous expression within 48 to 53 h in cattle, demonstrating that presence of a viable CL suppressed estrous expression. Subsequently, injections of progesterone blocked estrous expression and luteal formation in cattle (Ulberg et al., 1951). Briefly, the evolution of modern-day protocols for synchronizing estrus and ovulation has proceeded as follows: 1) administration of exogenous progesterone or progestins (e.g., medroxyprogesterone acetate; MAP) until spontaneous luteolysis occurred, 2) combination of short-term progestins (e.g., norgestomet) and estradiol, 3) administration of $\text{PGF}_{2\alpha}$ to induce luteolysis, 4) short-term progestin treatment plus $\text{PGF}_{2\alpha}$ administration at the end of progestin treatment, and 5) combination of a short-term progestin, $\text{PGF}_{2\alpha}$, and GnRH to synchronize follicular waves and more precisely control the time of ovulation for purposes of inseminating at a predetermined time. Tremendous progress has been made toward development of protocols that permit the control of estrous expression and ovulation in cattle with acceptable pregnancy rates and has been reviewed elsewhere (Odde, 1990; Lauderdale, 2009; Wiltbank and Pursley, 2014).

THE PROBLEM OF ANESTRUS

Failure to express estrus by the start of the breeding season is one of the fundamental limitations to optimizing reproductive efficiency in domestic ruminants. In regards to puberty in sheep and cattle, the first ovulatory estrus is preceded by a gradual decrease in the negative feedback of estradiol as puberty approaches (Foster and Ryan, 1979; Day and Anderson, 1998) and the timing of puberty can be influenced by nutrition (Day and Anderson, 1998). At the University of Wisconsin, Lester Casida (Fred McKenzie's first PhD student) and his graduate students conducted a series of studies in postpartum dairy and beef cows investigating factors affecting the interval from parturition to estrus and ovulation as well characterizing changes in pituitary gonadotropins and ovarian function during the postpartum period (Casida, 1968). Since that time, we have gained significant understanding about the mechanisms associated with postpartum anestrus (Short et al., 1990) and the effects of nutrition (Randel, 1990), suckling (Williams, 1990), and

the cow\calf bond (Viker et al., 1989; Griffith and Williams, 1996) on postpartum interval length in cattle and the effect of seasonality on sheep reproduction (Legan and Karsch, 1980). Much of the preceding research findings have been translated into current management practices such as a body condition scoring system for cattle and methods of manipulating suckling frequency to enhance postpartum reproduction under drought conditions.

In prepuberal and postpartum sheep and cattle, the first ovulatory estrus is preceded by a short luteal phase (Lauderdale, 1986). Mechanisms associated with regulation of short-lived corpora lutea have been reviewed elsewhere (Garverick and Smith, 1986; Lishman and Inskeep, 1991). Characterization of the short luteal phase that precedes the first ovulatory estrus in prepuberal and anestrus cattle and sheep has had a major impact on reproductive management practices in these species. In peripuberal heifers and anestrus cows, progestin treatment (e.g., Controlled Internal Drug Release) is commonly used in estrous synchronization protocols to simulate the short luteal phase and induce establishment of normal estrous cycles.

THE PROBLEM OF EMBRYONIC AND FETAL MORTALITY

Prenatal mortality is a major cause of reproductive loss in mammals, including domestic ruminants. Therefore, understanding the mechanisms resulting in embryonic/early fetal mortality has been a research focus during the past 50 yr. Nutrition, genetics, chromosomal abnormalities, sperm/oocyte quality, time of breeding, endocrine changes, uterine environment, immunological imbalances, stress, disease, and environmental factors (e.g., heat stress) can impact embryonic/fetal survival. Initial publications characterizing the incidence of embryonic loss and possible contributing factors in domestic animals were published 100 yr ago (Hammond, 1914; Robinson, 1921). Approximately 40 yr later, L. E. Casida (1953) stated the following: "The success of an embryo in its development may depend on its surviving a series of hazards and these hazards may be accidents of development or they may be genetically determined weaknesses in certain developmental steps. The fact that there are maternal differences in embryonic death rate gives emphasis to the study of the maternal environment itself even though as a rule only a part of the embryos perish. The chief variability in the character would appear to be in the maternal

threshold for bringing about its expression, i.e., for converting the accidents or the weaknesses of development into embryonic fatalities." These early studies which focused on the interaction between the uterine environment and conceptus development lead to investigation of uterine and conceptus gene products required for pregnancy success.

A thorough review of embryonic mortality in domestic species was published in 1994 (Zavy and Geisert, 1994) and provided a summary of knowledge up to that time. Estimates of early embryonic loss range from 8% to 30% before day 30 of gestation in cattle and sheep (Ayalon, 1978; Thatcher et al., 1994; see Nancarrow, 1994; Zavy, 1994), with later fetal losses ranging from 4% to 14% in cattle (see Pohler et al., 2015). Early embryonic loss also occurred when good quality blastocysts were transferred into recipient females at the appropriate stage of the cycle (see Hasler, 2014). The majority of embryonic loss is generally believed to occur early during the second to third week of pregnancy, which coincides with conceptus expansion, MRP, and placentation. Initial studies characterizing the incidence of early embryonic mortality in ruminants required serial collection of reproductive tracts at an abattoir following breeding. However, Ginther's pioneering work with real-time ultrasonography of the bovine reproductive tract (see review Ginther, 2014) provided a method for accurately monitoring embryonic/fetal development and survival beginning around day 26 or 27 of gestation (Pierson and Ginther, 1984; Kastelic et al., 1988; García et al., 1993). Gray-scale ultrasonic imaging was available in the 1980s and now Color-Doppler ultrasonic imaging has increased our knowledge of uterine development, uterine blood flow, luteal blood flow, and embryonic development and survival.

Early Pregnancy Diagnosis

A limitation of real-time ultrasonography for detection of pregnancy in ruminants is that the embryo and/or amniotic fluid vesicle are not reliably visible until day 26 to 27 of pregnancy. Detection of pregnancy or embryonic loss before return to estrus (day 17 to 21) has been a major objective for studying embryonic mortality and for reproductive management. Measurement of circulating or milk concentrations of progesterone was utilized to determine the absence or presence of a viable CL near the normal time of luteolysis (see Sasser and Ruder, 1987). Although concentrations of progesterone can be an indirect marker of embryo survival and establishment of pregnancy, variability in the

time of luteolysis (e.g., 2 vs. 3 follicular waves) and embryonic loss after day 21 of gestation, decreases the accuracy of the test. In addition to progesterone concentration, Doppler ultrasonography is currently being investigated to characterize luteal blood flow between day 18 and 21 of pregnancy, to provide an earlier indirect test for return to estrus (Scully et al., 2015).

Butler et al. (1982) were the first to isolate pregnancy-specific protein B from placenta of cattle and develop a RIA for early pregnancy detection in cattle (Sasser et al., 1986) and sheep (Ruder et al., 1988). Pregnancy-specific protein B originates from binucleate trophoblast cells, which also produce placental lactogen (see review by Wallace et al., 2015). Pregnancy-specific protein B is a member of a large gene family of placental expressed proteins released into the blood throughout pregnancy and are now named pregnancy-associated glycoproteins (PAGs; Wallace et al., 2015). Detection of PAGs in circulation has been utilized as an accurate pregnancy test, beginning around day 28 post breeding, and is commercially available. Pregnancy-associated glycoproteins are not only useful for the detection of pregnancy after day 28 of gestation, but may prove to be a possible marker for detecting females with a high probability of losing a pregnancy after day 28 (see Pohler et al., 2015).

Attempts to Decrease Embryonic Mortality

Wiltbank et al. (1956) proposed that early embryonic loss might be due to a progesterone deficiency or luteolysis in the presence of a viable conceptus. Researchers have attempted to improve embryonic survival in cattle by providing supplemental progesterone or extending luteal life span following administration of hCG, GnRH agonists, or interferon-tau (IFNT; see Thatcher et al., 1994). Early studies focused on administering recombinant bovine interferon- α -1 (rbIFNA) to sheep and cattle following breeding to mimic conceptus production of IFNT. Treatment of cows with rbIFNA extended the interestrous interval to 27 d; however, embryonic survival was decreased, possibly due to hyperthermia induced by rbIFNT. Although rbIFNA treatment in cattle was negative, improvements in pregnancy rate of sheep were more positive (Schalue-Francis et al., 1991).

Attempts to reduce early embryonic loss by supplementing with progesterone have been studied since the 1950s (Wiltbank et al., 1956); however, this approach has produced mixed results (Thatcher et al., 1994). Discovery and translational

studies have provided insight into when progesterone treatment may be beneficial for improving conceptus growth and survival in cattle. Embryo transfer studies in sheep and cattle indicated that establishment of pregnancy required a tight synchrony between the stage of embryo and uterine development (Moore and Shelton, 1964; Rowson and Moor, 1966). A positive association between circulating concentrations of progesterone and early conceptus growth in both the ewe (Wilmut and Sales, 1981; Lawson and Cahill, 1983) and cow (Garrett et al., 1988a) is generally believed to be due to stimulation of endometrial growth factors following the downregulation of uterine epithelial progesterone receptors (Forde et al., 2011; Spencer et al., 2017). The requirement of uterine glandular secretion for conceptus growth and survival was demonstrated in the ovine uterine gland knockout model, where neonatal uterine gland development was inhibited following progestin administration (Spencer, 2014). Furthermore, supplementation of progesterone after ovulation advanced conceptus growth in ewes (Satterfield et al., 2006).

In cattle, progesterone administration before the endogenous rise in luteal progesterone shortened the interestrous interval (16 to 17 d; Garrett et al., 1988b), advanced conceptus growth (Garrett et al., 1988a; Forde et al., 2011), and advanced the uterine receptivity such that older embryos could be transferred successfully (Geisert et al., 1991). Consequently, conceptus growth was influenced by the timing of the first plasma rise in progesterone concentration (normally day 4 to 5 in cattle). Low circulating concentrations of progesterone on day 4 to 5 of pregnancy have been associated with slower conceptus development, which could be accelerated by administration of progesterone (see Spencer et al., 2016). A meta-analysis of over 80 publications utilizing progesterone therapy to improve fertility in cattle indicated that progesterone treatment on day 3 to 6 of pregnancy was beneficial in cows of lower fertility (Yan et al., 2016).

CONCEPTUS DEVELOPMENT AND ESTABLISHMENT OF PREGNANCY

The description of the first ruminant conceptus was made by a physician, William Harvey, who dissected the uteri of deer harvested by King Charles in 1651. Harvey was influenced by the prevailing dogma at that time and expected to find a structure in the uterus similar to the yolk of an avian egg (like an ovarian follicle). However, instead he

found a “yellowish, friable, purulent matter” in the uterus, which sounds similar to an elongated conceptus (Short, 1977).

An excellent analysis of the history of the mammalian egg and conceptus development is provided by Betteridge (1981). Winters et al. (1942) accurately described the development of the bovine conceptus, whereas development to the blastocyst was described by Hamilton and Laing (1946). Although M. C. Chang is widely recognized for his studies in fertilization, he published one of the earliest serial descriptions of bovine conceptus development and implantation, which he collected from cows at an abattoir in San Antonio, TX (Chang, 1952). Greenstein et al. (1958) provided a more complete evaluation of post-elongation bovine development and the histological changes during placental formation and attachment to the uterine surface. Subsequently, Rowson and Moor (1966) provided a description of the ovine conceptus. Later cytochemical and ultrastructural studies provided more detailed images of the cellular events involved with conceptus attachment and placenta development (Guillomot et al., 1981; Guillomot and Guay, 1982; Wooding et al., 1982; Wang et al., 2009; see reviews King et al., 1982; Betteridge and Flechon, 1988; Guillomot, 1995).

Assheton (1906) first suggested that binucleate cells may be associated with ovine placentome formation and Amoroso (1952) reported that chorionic binucleate cells migrated into the uterine epithelium. Later bovine placental development (30 d) was first described by Hammond (1927) with detection of binucleate giant cells in the chorion reported by Wimsatt (1951). Work by G. J. King and F. B. P. Wooding provided some of the most stunning histological evaluations of attachment and placental development in ruminants (King et al., 1981; Wooding, 1982, 1984; King and Atkinson, 1987). Formation of binucleate cells and their migration to form the syncytial plaques with the uterine surface epithelium has been reported (Wooding, 1980; Wooding and Wathes, 1980). Binucleate cell migration provides a mechanism to deliver placental lactogen, placental growth hormone, PAGs, and prolactin-related protein as well as steroids to the maternal system (Bazer et al., 2012b). Release of binucleate cell growth factors and steroids may play a role in uterine development, nutrient transport, uterine blood flow, and immunoregulation which continues to be investigated today.

Uterine blood flow was intensely investigated during the late 70s and early 80s (Ford, 1982, 1995) and is essential for continued fetal growth and

survival, which can be directly affected by environmental conditions (Roman-Ponce et al., 1978a, 1978b; Redmer et al., 2004). With increasing global temperatures, management strategies to alleviate detrimental effects of heat stress on early embryonic survival, fetal growth, and lactation remain a primary focus in dairy management systems. Growth of the placenta, placenta fluid changes, and maternal endocrine patterns during later stages of pregnancy were investigated to understand growth of the bovine (Eley et al., 1978, 1981) and ovine (Bazer et al., 2012a) fetus to term.

The rapid growth of the ruminant conceptus during the second week of gestation is well documented (Rowson and Moor, 1966; Betteridge and Fléchon, 1988; Spencer et al., 2017). Grosser (1927) and Amoroso (1952) understood the significance that endometrial products absorbed by the placenta (i.e., histotroph) have in conceptus development and survival. Meyer (Amoroso, 1952) proposed the term “embryotrophe” to describe endometrial products supplied to the embryo. However, technologies had not been developed to isolate and determine the specific endometrial and conceptus factors involved at the time. The importance of progesterone in timing uterine synchrony with embryo transfer (Rowson and Moor, 1966), presence of uterine glands (Spencer, 2014), and formation of trophoblast papillae over the uterine glands during conceptus development (Wooding et al., 1982) indicated the important role of the endometrium in embryonic survival.

Bazer et al. (1969) and others initiated the search for uterine factors involved with conceptus development and implantation. Early experimental approaches utilized radiolabeled amino acids (i.e., methionine) in culture, analysis of conceptus-conditioned media via 2D-PAGE analysis and column chromatography to identify secreted uterine embryotrophic and conceptus factors involved with establishment of pregnancy in ruminants (Bazer et al., 1979; Bartol et al., 1985a, 1985b; Godkin et al., 1985; see Roberts and Bazer, 1988). Today, techniques in genomics, transcriptomics, proteomics, metabolomics, glycomics, and bioinformatics have improved our ability to identify the multitude of gene products and their pathways associated with conceptus attachment, growth, and the establishment of pregnancy (Brooks et al., 2016; Ribeiro et al., 2016; see Forde et al., 2015; Spencer et al., 2017). Furthermore, utilization of CRISPR/Cas 9 gene editing has allowed researchers to more precisely examine the physiological role of genes expressed by the conceptus (Brooks et al., 2015;

Whyte et al., 2018). Recently, the discovery of extracellular vesicles containing microRNAs, which can regulate gene expression during the establishment of pregnancy, has stimulated a new area of research (Burns et al., 2016; Pohler et al., 2017).

In 1969, Roger Short coined the term “Maternal Recognition of Pregnancy” to describe the conceptus signal that is required for maintenance of luteal function beyond the normal length of the estrous cycle (Short, 1969). Following the discovery that $\text{PGF}_{2\alpha}$ is the uterine luteolysin in ruminants (McCracken et al., 1972; McCracken et al., 1981; Schramm et al., 1983), investigators focused on identifying conceptus factors responsible for maintenance of luteal function. Roberts (2017) recently was the editor of a series of papers concerning the 30-yr history of the discovery of IFNT and its biological role. In addition, Bazer and Thatcher (2017) provided an excellent review of the discovery of IFNT, which will be briefly summarized below. Embryo transfer studies in sheep (Rowson and Moor, 1966) and cattle (Betteridge et al., 1980) demonstrated that the maternal environment recognized the conceptus on day 13 and 16 post-estrus, respectively. These results were confirmed by studies that showed that maintenance of the CL occurred only when conceptuses were flushed from the uteri of pregnant cows on day 17 (Northey and French, 1980) and pregnant sheep on day 13 (Rowson and Moor, 1966). Intrauterine infusion of conceptus homogenates lengthened the interestrous interval of cows (Northey and French, 1980) and sheep (Rowson and Moor, 1967) and the biologically active factor was reported to be heat-labile and sensitive to protease degradation. These studies provided the initial evidence that ruminant conceptuses produced a protein factor(s) to inhibit luteolysis.

Martal et al. (1979) reported that the conceptus protein, associated with luteal maintenance, was detected in extracts of sheep conceptuses on day 14 to 15 but not day 21 to 25 and was named “trophoblastin.” Following Martal’s initial publication, the laboratories of Fuller Bazer, Michael Roberts, and William Thatcher, at the University of Florida, identified the conceptus factor by employing radiolabeled amino acids and examining ovine conceptus-conditioned medium. This experimental approach improved the ability to detect de novo synthesis of secreted proteins from conceptuses. The conceptus protein was first named Protein X and later renamed ovine trophoblast protein one (Godkin et al., 1982) and was able to extend the interestrous interval of cyclic ewes (see Bazer and

Thatcher, 2017). Ovine trophoblast protein was cloned and determined to be a unique member of the Type 1 interferon alpha family (Imakawa et al., 1987; Stewart et al., 1987). The official name of the ovine and bovine conceptus protein is IFNT. Conceptus IFNT has antiviral, antiproliferative, and immunosuppressive activity typical of interferons (Roberts et al., 1992).

The antiluteolytic role of IFNT during early pregnancy has been intensively investigated. John McCracken and others developed a model for regulation of uterine $\text{PGF}_{2\alpha}$ secretion in nonpregnant sheep (McCracken et al., 1984). In this model, elevated circulating concentrations of progesterone induce downregulation of progesterone receptors in the uterine epithelium thus allowing upregulation of endometrial estrogen receptors. Estradiol, presumably from the ovary, binds to endometrial estradiol receptors and upregulates endometrial oxytocin receptors. Circulating oxytocin, initially from the posterior pituitary and subsequently from the CL, binds oxytocin and induces pulsatile $\text{PGF}_{2\alpha}$ secretion to induce luteolysis. The local action of IFNT inhibits endometrial estrogen receptor expression and subsequent $\text{PGF}_{2\alpha}$ secretion (see Spencer and Hansen, 2015). More recently, IFNT has been shown to have an endocrine role in MRP (Hansen et al., 2010).

Prostaglandins play an important role in ruminant conceptus development as inhibition of PG synthesis inhibits conceptus elongation during the establishment of pregnancy (Spencer and Hansen, 2015). Early research indicated that conceptus and endometrial production of PGE_2 could temporarily maintain the CL in ruminants (Henderson et al., 1977; Pratt et al., 1979) and the studies of Charley Weems laboratory indicated the possible role of PGs, specifically of PGE_2 , in the establishment of pregnancy (Weems et al., 2006). The reciprocal interaction between secretory products of the conceptus and maternal endometrium termed the “servomechanism” for the establishment of pregnancy (Spencer et al., 2007) continues to be a focus of research to improve embryonic development and survival in ruminants.

EMBRYO TRANSFER

The first successful transfer of embryos was in rabbits (Heape, 1891) and it took over 40 yr to produce the first live birth of a lamb (Warwick et al., 1934) and calf (Willett et al., 1951) by embryo transfer. Muttter et al. (1964) produced the first calf born following nonsurgical embryo transfer.

These landmark studies laid the foundation for improving genetic merit of ruminants through embryo transfer. This technology was advanced in the 1970s to 1990s and the historical progress of embryo transfer has been described previously by Keith Betteridge (Betteridge, 1981, 2000, 2006, 2014). Progress and influence of embryo transfer in cattle was featured on the cover of Science magazine in 1981 (Seidel, 1981). During the last 50 yr, efforts to improve the embryo transfer technique resulted in advancements in other technologies including methods of estrous synchronization, superovulation, nonsurgical embryo recovery and transfer equipment, embryo culture media, methods of evaluating stage of embryo development and quality, and cryopreservation (Hasler, 2014). Many of the advances in embryo transfer and the transfer of this technology to the livestock industry were contributed by the work of Keith Betteridge, Peter Elsdon, Bob Foote, John Hasler, L. E. A. (Tim) Rowson, Bob Moor, George Seidel, Steen Willadsen, and Ian Wilmut (see Foote, 1987; Hasler, 2014; Seidel, 2015).

IN VITRO FERTILIZATION, CLONING, TRANSGENICS, AND GENE EDITING

The first calf resulting from an in vitro fertilized oocyte and in vivo capacitated sperm was born in 1981 (Brackett et al., 1982). Fukuda et al. (1990) were the first to produce a calf born following transfer of blastocysts formed from in vitro matured oocytes that were fertilized and cultured in vitro. Neal First was one of the early pioneers of in vitro fertilization in cattle (see First, 1987). In the 1980s, embryo splitting was developed as a method of cloning ruminants (Fehilly and Willadsen, 1986). Johnson et al. (1995) produced quadruplet calves from a 4-cell embryo; however, the limited number of clones generated from this technique provided impetus for the development of other cloning methods. Research from Neal First's lab and others (see Foote, 1987; Seidel, 2015) established methods to transfer nuclei to oocytes to create clones. Transfer of nuclei from 8 to 16 cell embryos resulted in the live birth in sheep (Willadsen, 1986) and cattle (Prather et al., 1987). These studies clearly laid the foundation for the historical birth of "Dolly" following somatic cell nuclear transfer of an adult donor cell (mammary cell line; Wilmut et al., 1997). This stunning achievement accelerated advancements in somatic cell nuclear transfer and the use of genetically modified donor cells. However, 20 yr after the birth

of Dolly, the impact of her birth has done more to stimulate advances in stem cell biology than cloning of domestic ruminants.

The first transgenic mammals were mice generated from oocytes microinjected with metallothionein-growth hormone genes (Palmiter et al., 1982). Since that time, a variety of techniques have been used to introduce foreign DNA into cells including retroviruses, pronuclear microinjection, and embryonic stem blastocyst injection. Utilization of homologous recombination and somatic cell nuclear transfer has been used to produce transgenic cattle (Cibelli et al., 1998), sheep (Schnieke et al., 1997), and goats (Keefer et al., 2001). More recently, development of nucleases that provided greater precision in cleaving DNA such as zinc finger nucleases, TALENS and CRISPRs have improved the rate and efficiency of producing transgenic animals (Wells, 2016). Engineering of the mammary gland of cows to be resistant to *Staphylococcus aureus* infection is one example of using biotechnology to improve animal health (Wall et al., 2005). The development of CRISPR gene-edited pigs resistant to porcine reproductive and respiratory syndrome virus has provided a blue print for improving animal health (Whitworth et al., 2016). As with the AI technique, the goal of genetic improvement was a driving force for the research underlying development of the embryo transfer technique, in vitro fertilization, and cloning.

CONCLUSION

Over the past 50 yr we have learned a lot about physiological, cellular, and molecular mechanisms controlling reproduction in domestic ruminants. However, a lot can be learned about the process of scientific discovery by observing how the field of reproductive physiology has evolved. A few important principles of scientific discovery include the following: 1) understanding a field of biology frequently begins with a descriptive phase followed manipulation of a system to understand the underlying physiological, cellular, or molecular mechanisms, 2) research is essentially problem-driven whereby an investigator identifies an economically important problem and initiates a series of experiments to alleviate the problem, 3) a research career is spent contributing links in a continuous effort of exploration that involves others, which leads to the next question, 4) collaboration has been essential to progress. As technology becomes increasingly complex the development of research teams has become essential for advancement, 5) read outside

your area of expertise, 6) need to be observant and know when to pursue an unexpected observation. Remember Chris Polge and the discovery of glycerol as a cryoprotective agent, 7) remember that variation in a data set may be informative (e.g., frozen semen—Chris Polge), 8) look for the unexpected, but be cautious about the “expected”—strive to remain objective! and 9) finally, remember what Carl Hartman said—“Research Spells Fun” (Hartman, 1967).

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