

## **THE MAKING OF A CALF**

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### **Introduction**

Have you ever uttered a few “BAD” words when told by the veterinarian that some of your cows, you saw bred by the bull, weren’t pregnant? After calming down, did you ask or ever wonder why they didn’t get pregnant? Was it the cow’s or the bull’s fault, did they consume some type of toxic plant, is there a problem with their nutrition or minerals, or did the estrus synchronization protocol or AI technician not perform to par? The list of possibilities for why cows don’t get pregnant can go on and on. Some of the reasons will be covered in the conference presentations or in the accompanying proceedings. However, only after a thorough review of the reproductive process and those factors that may impede success will you begin to know if any of these are problematic in your herd. If they are, you are not likely alone. In Tennessee, only 76 to 80% of cows wean a calf each year. Management may be a contributive factor as to why almost a quarter of cows do not wean a calf. In cases where it is, simple corrections often go towards improving calf crop. Considerate of all possible factors, they can add up quickly to yield a great calf crop or one falling below expectations. A producer’s ultimate goal should be to have a 90+% calf crop every 365 days. Most in attendance probably reach this goal in most years; however, may not be without a few stories of their own where the veterinarian said the word “OPEN” too many times.

It’s probably fair to say that the complexity and challenges of making a calf are not often thought of by a producer when they hear the word “OPEN”. Yet they exist and are quite complex even for those with more advanced training in the area of reproductive physiology. Because of this, an important first step towards not only achieving but maintaining a calf crop >90% is dependent on gaining a basic understanding of the process of making a calf. Others will describe/discuss the physiological principals of the estrous cycle and important reproductive hormones, insemination-related factors affecting fertilization, bull management, and the impact of grazing tall fescue among other important factors having a major influence on reproductive success. The intent of this article is to highlight challenges most central to the making of a calf. While few would dispute it “takes two” (i.e., egg from the cow is fertilized by a spermatozoon from the male; see Figure 1), the challenges related to the “pilgrimage” of the gametes to the site of fertilization and the production of an embryo competent to progress throughout pregnancy and result in a healthy calf are often overlooked or taken for granted.

### **Reproductive Anatomy**

We start our discussion in an area with which most in attendance are likely familiar, the reproductive anatomy of the female. Before selecting a heifer as a replacement or wondering what went wrong with the cow the veterinarian just called “OPEN”, it is important to know if they have the “machinery” with functional parts to produce a live, healthy calf. This would not be the case if the heifer was born twin to a bull calf (i.e., freemartin). To start, are all the parts there in that replacement heifer? Are the vulva,

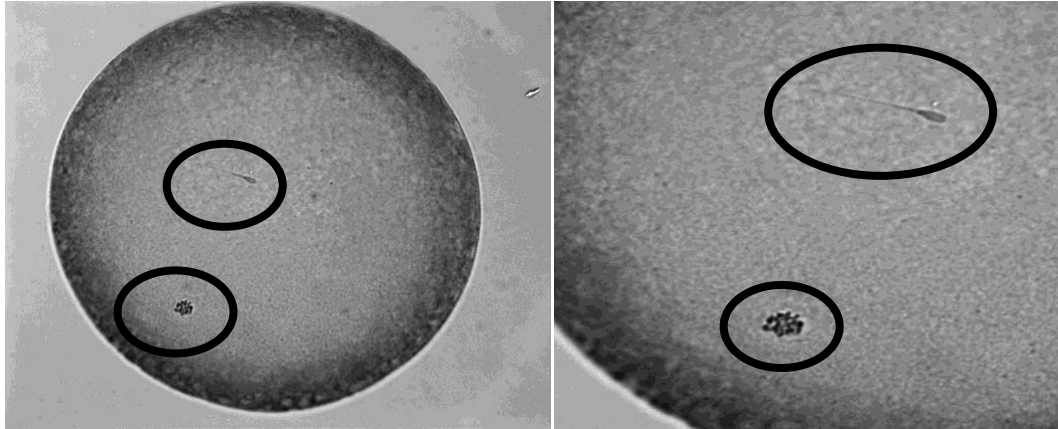


Figure 1. Bovine oocyte with maternal DNA (lower circle) fertilized by an intact sperm containing paternal DNA (upper circle).

vagina, cervix, uterus, oviducts and ovaries (Figure 2) all there and working properly? If this is a cow, was any damage done to the reproductive tract the last time she calved?

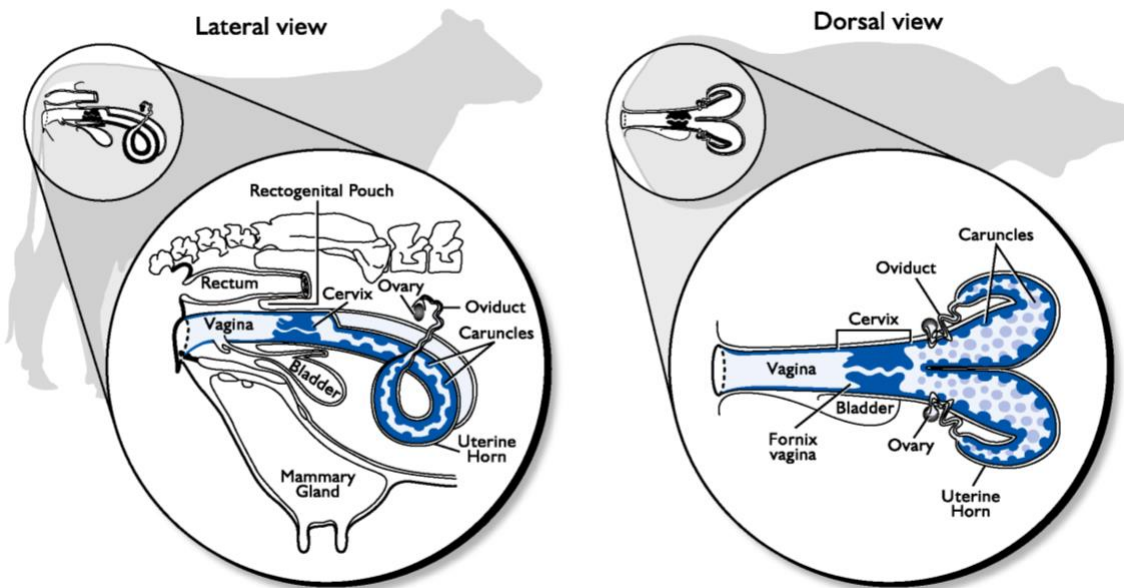


Figure 2. Schematic illustration of the female reproductive tract in the bovine. From *Pathways to Pregnancy and Parturition*, 2<sup>nd</sup> revised ed., Copyright 2005. Permission by Current Conceptions Inc.

Are the ovaries functional and the uterine environment hospitable for embryo development and maintenance to term? To determine if the machinery is there with all required parts is a straightforward procedure involving palpation of the reproductive tract (and most of us can assume all is in order without palpation). However, determining if all parts are functioning properly is a different challenge without any simple answers or tests. To this end, if machinery is present, one is to assume all is in working order until the dreaded word “OPEN” is spoken. Some in attendance may perform scoring of the reproductive tract in their replacement heifers which provides some

information about the reproductive status of these animals. Very seldom is this performed in mature cows unless they are either donors for embryo production or recipients for these embryos.

Regarding assessment of the bull, most in attendance have a breeding soundness examination (BSE) performed. During this examination, the veterinarian will make sure that the testes, scrotum, epididymis, vesicular glands, and penis are there without abnormalities (Figure 3). If the bull can walk, see, is in good body condition, and has all

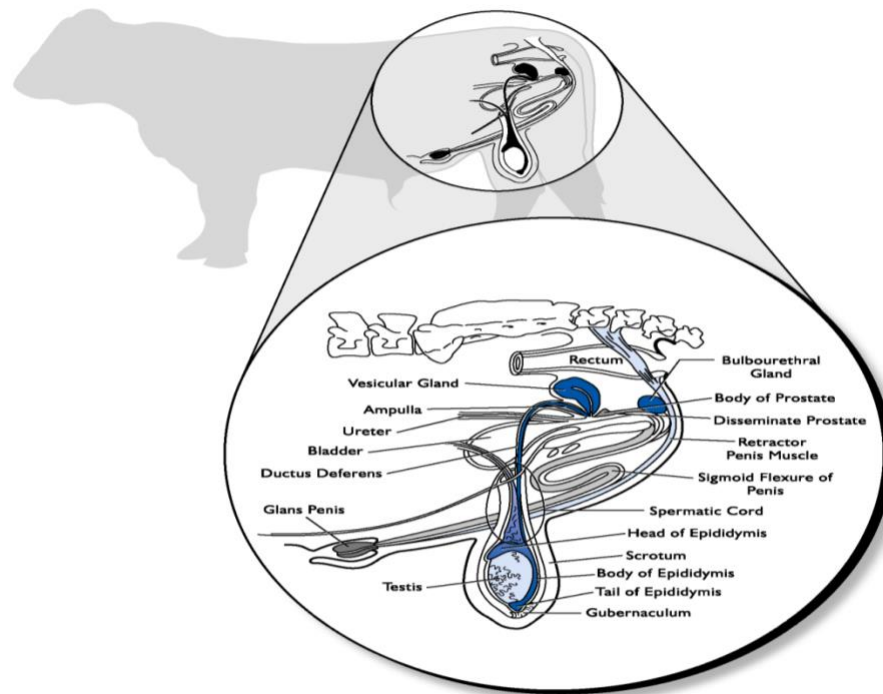


Figure 3. Schematic illustration of the male reproductive tract in the bovine. From *Pathways to Pregnancy and Parturition*, 2<sup>nd</sup> revised ed., Copyright 2005. Permission by Current Conceptions Inc.

the necessary “machinery” and parts for sperm production and release, then they’ll evaluate the semen. But one must remember that the evaluation is only good for that day and does not predict the bull’s desire to breed cows (libido). If the bull gets sick or has an infection (elevated temperature) or his testes are damaged during a fight or stepped on during the ride home, all bets are off. So recognize that a breeding soundness exam while informative is not an absolute predictor of fertility. But to date, it is the “best” test available for assessing breeding soundness and potential fertility of the bull.

### **An Oocyte’s Journey**

The journey of the oocyte to conclude in a term pregnancy involves more than just departing from the ovulatory follicle and taking the “short trip” to the middle part of the oviduct. Unlike sperm, all the oocytes (eggs) that will ever be produced were made while the female was in the early stages of fetal development while in utero. Maximal numbers are actually achieved before the female is born. At birth, heifers typically have about a million oocytes with the majority located in ovarian “egg nests”. If one assumes that a beef cow is capable of producing 10 calves, clearly

the vast majority are not utilized for the purpose of making a calf. Rather, the majority at some point or another degenerate/die as a result of follicle turnover. While there is not a test or assay to determine which oocytes will result in a calf, clearly many more than 10 are capable, since calves are routinely produced from oocytes obtained from superovulation procedures and abattoir-derived ovaries.

It is important to note that individual oocytes soon after formation become surrounded by a single layer of follicle cells. This effectively forms a follicle in its simplest form and is a first important step in a series for building the “house” (the follicle) for the very important “occupant” (the oocyte). Most in attendance may know that follicle growth is a very dynamic process and occurs in waves throughout the female’s life in the bovine (will be discussed by Dr. Smith). While several may be recruited, not every follicle will develop to a size capable of ovulating. In fact, all but one or two follicles will degenerate (die) through a type of cell death known as atresia, taking the oocyte with it (Figure 4).

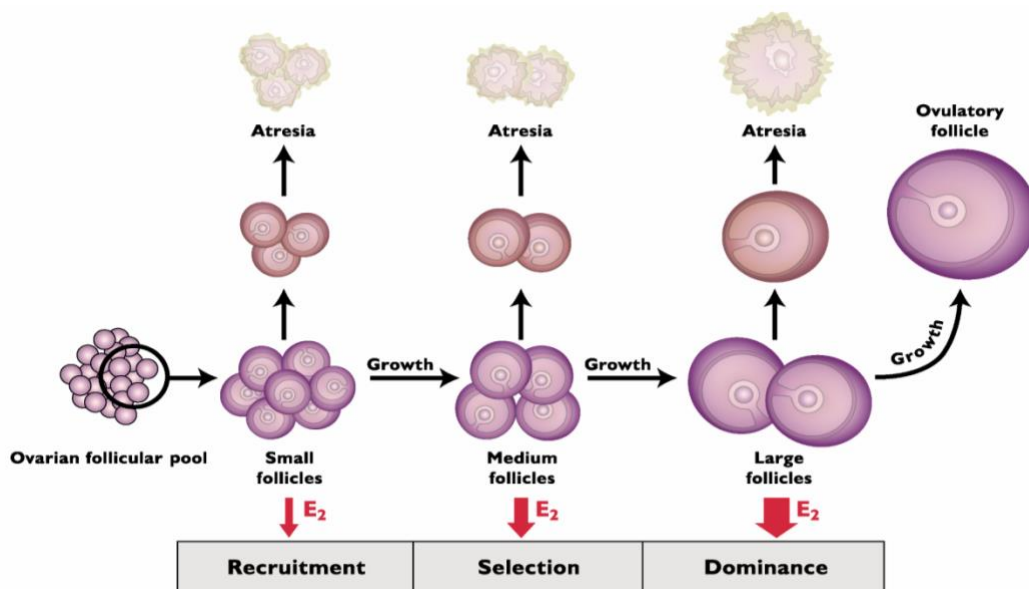


Figure 4. Schematic illustration of follicular development in the bovine. From *Pathways to Pregnancy and Parturition*, 2<sup>nd</sup> revised ed., Copyright 2005. Permission by Current Conceptions Inc.

An example we find helpful to understand the concept of follicular-oocyte development is college football recruiting. The coach recruits 15 to 20 high school football players each year for the offensive line. Of these, 7 to 10 will be selected to attend the University of Tennessee but only 5 starters are needed for the offensive line (center, 2 guards, and 2 tackles) and maybe only 1 or 2 of these from each class (freshman, sophomore, junior, senior) will actually start. Of these offensive starters on the line, only 1 will be an All-American which will correspond to the ovulatory follicle-oocyte. Thus, you start with several follicles-oocytes (high school football recruits) and end typically with one follicle-oocyte complex (All-American) that has the potential to become a calf.

Besides its important role in providing the “house” for the oocyte, the follicle is also important for hormone production. Relevant for this topic is its role in estradiol production sufficient to cause

expression of estrus and induce the LH surge. While most are familiar with LH's role in causing ovulation, fewer recognize its importance for stimulating the oocyte while contained within the ovulatory follicle to resume meiosis (i.e., mature in preparation for fertilization). Simply put, the oocyte contained within the ovulatory follicle, before the LH surge, has too many copies of chromosomes. The LH surge effectively "forces" the oocyte to extrude (get rid of) the extra copies of chromosomes and initiates other important changes. This process, termed oocyte maturation, coincides with estrus in the cow and is completed before rupture of the ovulatory follicle, effectively preventing the spermatozoon waiting patiently in the oviduct from fertilizing the oocyte before its ready. Because of the complexity of the changes occurring during this time, the maturing oocyte is very susceptible to environmental stressors. In particular, those stressors resulting in elevated body temperatures (e.g., heat stress, ingestion of toxic plants, illnesses or others). Depending on the severity of the stressor, reductions in fertility related to direct effects on the oocyte are a likely outcome.

So the journey for the oocyte resulting in a term pregnancy begins while the heifer is in utero during gestation but may not be completed until the animal/oocyte is 8 to 12 years of age. Thus, the oocyte must wait its turn. Furthermore, the signals necessary to identify that it is time to start developing are currently unknown. Recognize however that several factors can change these patterns of follicular-oocyte development and will be discussed throughout these presentations and proceedings. How does nutritional stress alter these growth/selection patterns and requirements? What about handling/facility stress during timed insemination on follicle ovulation or oocyte maturation? These are questions that may be answered during your time in Nashville.

### **The Sperm's Journey**

Compared to the oocyte's short trip in terms of distance traveled from the ovulatory follicle to the midsection of the oviduct, the spermatozoon (a single sperm cell) resulting in a pregnancy travels a distance more like going from the "West coast to the East coast, swimming across to the Atlantic Ocean to then travel across another continent". The first leg of its travels begins in the testes. On this part of the journey, which takes about 60 days, the sperm goes from being a type A spermatogonium, to a spermatid, to a spermatozoon (Figure 5) in the seminiferous tubule. Once the sperm becomes as we know it (i.e., has a head and a tail), it is released from its nurse cell (also known as a Sertoli cell) to begin its journey out of the testes. To this end, it travels through a series of major tubules which ultimately merge into a single one called the epididymis. This important "roadway" is where sperm acquire the ability to become motile and fertilize the oocyte. The tail of this structure is where the ejaculatory reserves of sperm reside. In other words, this is where sperm are held until they are released either through ejaculation (preferred route) or trickle out in the urine. Sperm are deposited into the vagina of the cow after natural mating or can be collected into a tube for subsequent evaluation and/or processing for eventual artificial insemination.



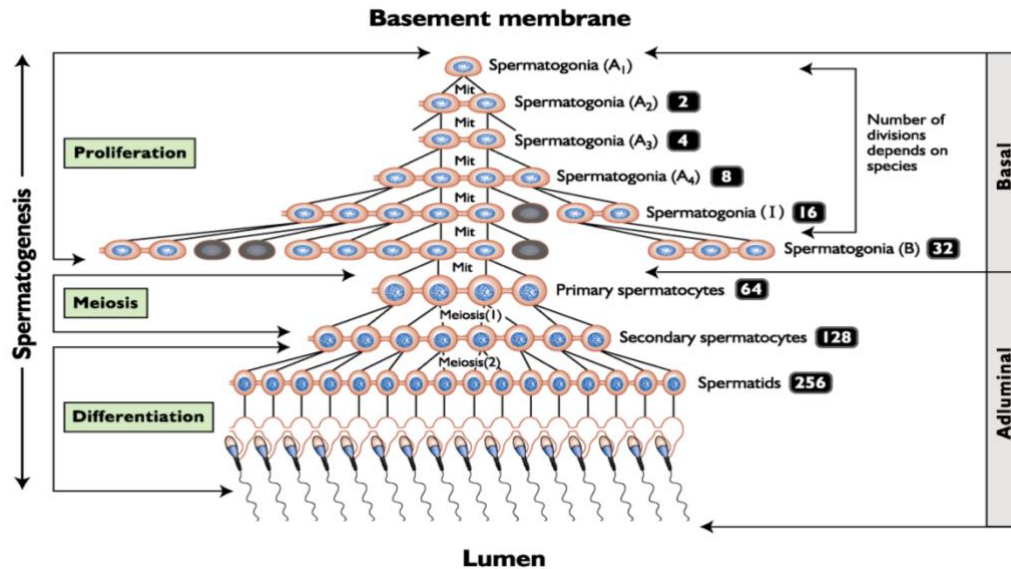


Figure 5. Schematic illustration of spermatogenesis in the bovine. From *Pathways to Pregnancy and Parturition*, 2<sup>nd</sup> revised ed., Copyright 2005. Permission by Current Conceptions Inc.

During the ejaculatory process, sperm are mixed with seminal plasma from the accessory sex glands. Some might compare this leg of a spermatozoon's journey to swimming in and across an ocean; whereas, deposition into the vagina may be likened to landing on a different continent. Most would contend that this is when a major portion of the fertilizing spermatozoon's journey begins. During the spermatozoon's travels to the site of fertilization (i.e., the oviduct), it is faced with numerous difficult/challenging obstacles. First it has to get out of the vagina (if deposited via natural mating) and make its way into/through the cervix (a major barrier). Thereafter, it has to make it into and through the correct uterine horn (the one connected to the oviduct that will eventually contain the oocyte). Keep in mind that during its travels, the spermatozoon is effectively "undressed" by its interactions with the different parts of the female's reproductive tract. Doing so is of utmost importance, otherwise the spermatozoon would not be able to bind to the oocyte. This process termed sperm capacitation does not happen quickly, so it is critical that sperm are deposited into the female's reproductive tract several hours before the oocyte is released from the ovulatory follicle. Assuming that it doesn't get "eaten" by an immune cell along the way, most sperm have the opportunity to take a break from their challenging travel soon after reaching the oviduct. It is here where they will attach to oviductal cells to get "recharged" as they wait for some signal that their "date" (i.e., oocyte) awaits their arrival. To this end, a limited number of sperm will be released from the oviduct very soon after ovulation and make their way to where the oocyte lies in wait. Of the billions ejaculated, you may be surprised to know that as few as 100 or so spermatozoa reach the site of fertilization. If nurse (cumulus) cells are still present on the oocyte, the spermatozoon must penetrate through these cells before being able to bind a specific receptor on the outer shell of the oocyte. Only after binding the receptor will the spermatozoon be able to make its way through this glycoprotein coat known as the zona pellucida. If it is successful in doing so, then it can bind/fuse with the oocyte. This process is referred to as fertilization, a critical step in the formation of a developmentally-competent embryo.

### The Making of an Embryo

Once the spermatozoon meets up with the oocyte and fertilizes it, a reaction occurs immediately thereafter to block any other sperm from entering. Some use an example of concrete forming around the edge to prevent others from visiting or courting the oocyte. Once inside, the sperm must be unpackaged to allow its genetic information to ultimately meet up with the oocyte's genetic information. After the two gametes' genetic information comes together, the resultant zygote, as it is now called, undergoes a series of simple cellular divisions. For example, the one cell embryo divides into two cells, two cells become four, four cells become eight and so on (Figure 6). You may also notice in Figure 6 that with each subsequent division of the early embryo the individual cells within (also known as blastomeres) decrease in size. Increasing cell number while reducing cell volume during these first few cleavage divisions is important as the cells comprising later stage embryos and in the adult are much smaller than the oocyte from which they were initially derived. Until the 8 to 16 cell stage, the embryo is primarily

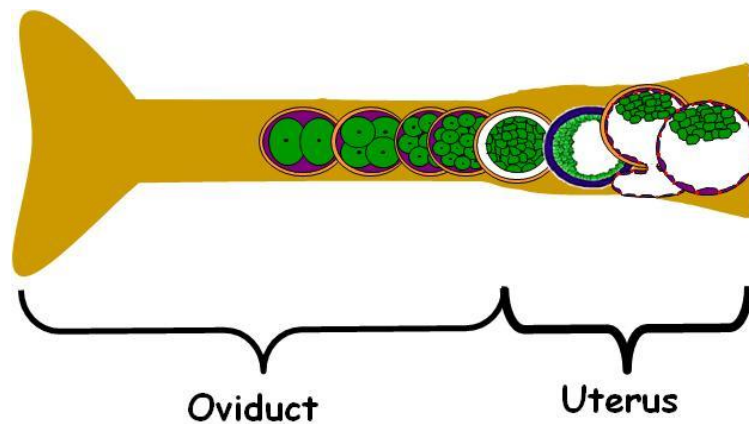


Figure 6. Schematic illustration of early embryo development in the bovine provided by Edwards, Hockett and Schrick.

under control of the maternal genome. In other words, those things contained within the oocyte's maternal cytoplasm inherited by the embryo and important for sustaining early development. So keep in mind that management considerations, as discussed over the next few days, should be optimal to ensure success during this time.

At the 8 to 16 cell stage, the embryo takes over control of its development through activation of its own genome. If this doesn't occur, an embryo will not develop further. The next developmental milestone in the life of an early embryo is the process of compaction. This occurs 5 to 6 days after fertilization and is the time period where the cells comprising the early embryo flatten down on one another and the embryo may be confused with an unfertilized oocyte by the less experienced evaluator. This allows the outer cells to differentiate into trophectoderm cells which ultimately results in formation of the placenta. The inner cells give rise to embryo/fetus. Tight junctions form between the trophectoderm cells so that when an enzyme starts pumping water into the middle of the embryo, a fluid-filled cavity is formed. This embryo with a fluid-filled cavity continues to grow (expands) to the point where it literally hatches out of its shell, the zona pellucida, approximately 8 to 9 days after insemination. After hatching, the bovine embryo elongates, ultimately resembling a thick spaghetti noodle. The more technical term for this stage is filamentous. During this time period, several changes and processes occur that will influence

success to term (Figure 7). Management changes should be minimized to reduce stress during this critical period of embryo development and will be discussed by others in subsequent presentations.

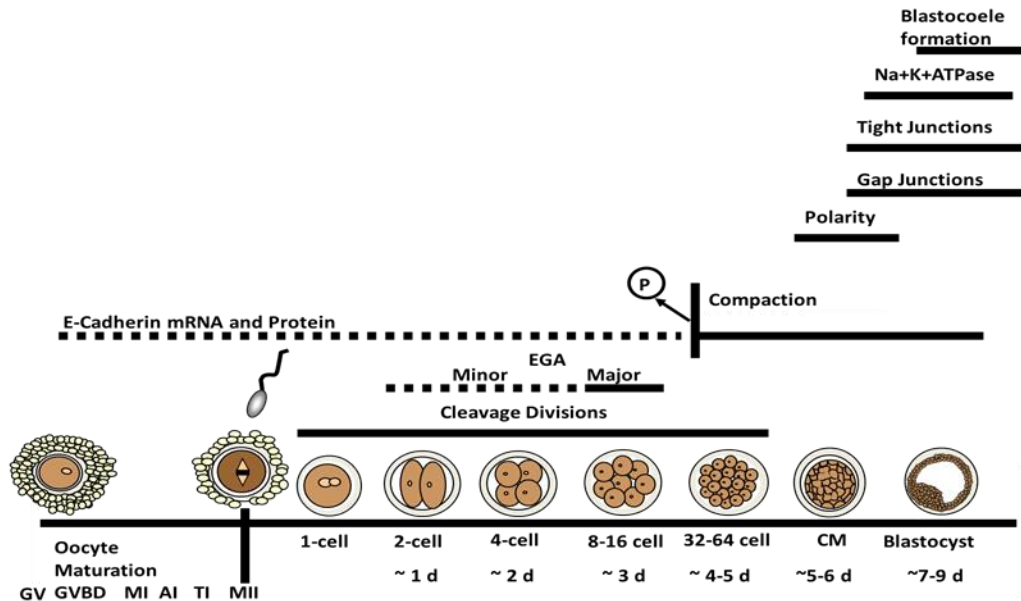


Figure 7. Schematic illustration of important developmental events initiated during early embryo development provided by Edwards and Schrick.

### Development of the Embryo/Fetus to Term

During expansion of the embryo, cells that subsequently produce the placenta form and continue in development throughout the initial stages of pregnancy. Around 16 days after breeding, the cells ultimately giving rise to the placenta in the filamentous embryo send a signal to the dam to inform her of the pregnancy. The basic gist of the message to the dam is to save the corpus luteum. For the corpus luteum (CL) to be “saved” for maintaining progesterone production, the hormone  $\text{PGF}_{2\alpha}$  must not be released in its pulsatile fashion. To this end, a protein called Interferon-tau is secreted by the placental cells which disrupts  $\text{PGF}_{2\alpha}$  pulses; thus, allowing for the CL to be maintained and progesterone production to continue. At approximately 22 days following breeding, a “heartbeat” should be visible within the embryo structure with ultrasonography. However, we do not recommend examining the embryo at this developmental stage because of its “fragile” state. Around day 29 to 32, attachment will occur in which the maternal (caruncles) and fetal (cotyledons) “connections” form for nutrient, gas and waste exchange throughout pregnancy.

Completion of embryo development and initiation of fetal development occur around 42 to 45 days of gestation. From there forward, little loss is expected to occur if disease and other issues are minimized. At the time of parturition, the fetus will actually initiate the process of calving by causing the release of glucocorticoids. The process includes release of relaxin to soften the cervix, since this is the birth canal of the calf, and release of  $\text{PGF}_{2\alpha}$  for luteal regression, and stimulating uterine contractions for expelling the fetus and the placenta after calving.

### Periods of Loss during Gestation

Arguments can be made as to when loss occurs during pregnancy in cattle. However, research would tend to indicate that the majority of loss occurs early during pregnancy involving several



factors. Again, if disease and management are held in check, fertilization success with AI or natural service (NS) is thought to be 90-95% (Diskin and Sreenan, 1980). So, if we start with 100 cows in heat at the right time and either a good technician or bull, we should have around 90 to 95 cows with embryos. However, calving rate to a single breeding is around 70 to 75%, so where does the loss occur? In a study of beef heifers with a single insemination at estrus, Dunne et al. (2000) observed that 68% of heifers had a viable embryo (in terms of size and morphology) at day 14 after breeding when they were sacrificed for the study. The remaining heifers not sacrificed were scanned by ultrasonography on day 30 after breeding with a 76% pregnancy rate and 72% calving rate. So the majority of loss had occurred before day 14 as reported by others and likely associated with a multitude of factors including genetic/chromosomal abnormalities, environmental insults, semen quality, and miscellaneous other factors.

### Summary

Several factors result in success or failure of your ultimate goal, a live calf at term. Several of these factors will be discussed this week with strategies that will hopefully improve your overall calf crop at weaning. Our final figure illustrates the complexity of reproduction in the cow through their reproductive lifespan (Figure 8).

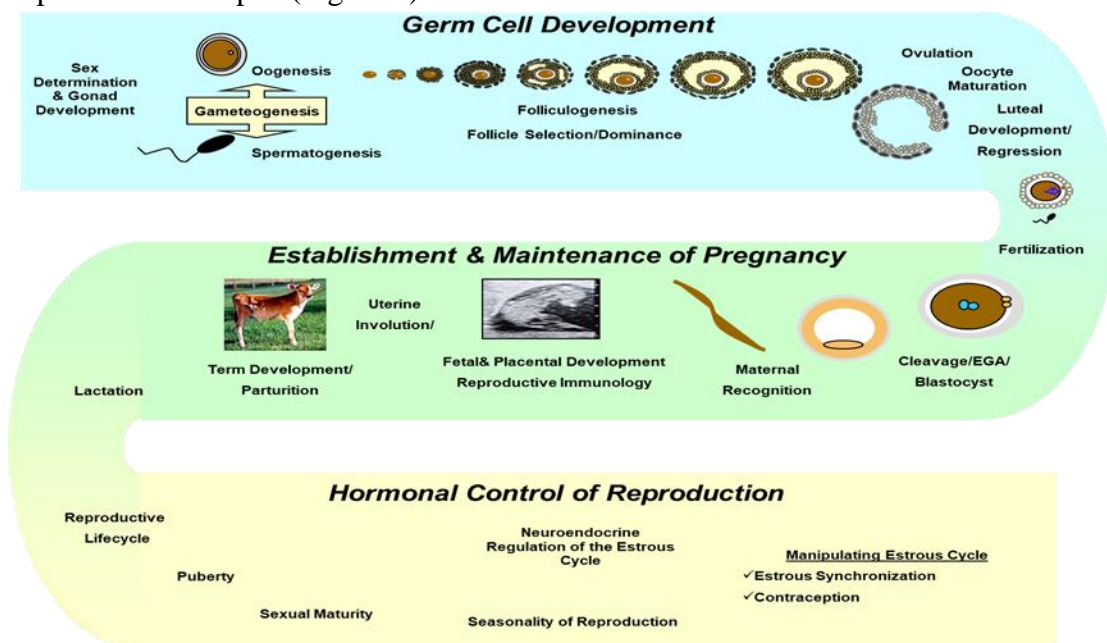


Figure 8. Schematic illustration of events occurring during the reproductive lifespan of a cow provided by Edwards.

Management is key, but increasing your knowledge base will allow for successful adaptation of changes sure to occur in protocols for artificial insemination, natural breeding and embryo transfer.

### References

- Diskin, M. G. and J. M. Sreenan. 1980. Fertilization and embryonic mortality rates in beef heifers after artificial insemination. *J. Reprod. Fertil.* 59:463-468.
- Dunne, L.D., M.G. Diskin, and J.M. Sreenan. 2000. Embryo and foetal loss in beef heifers between day 14 of gestation and full term *Anim. Reprod. Sci.* 58:39-44.

**Suggested Readings**

Senger, P.L. 2005. Pathways to Pregnancy and Parturition. 2<sup>nd</sup> Revised Edition. Current Conceptions Inc., Pullman, WA.

Bearden, H.J. and J.W. Fuguay. 1992. Applied Animal Reproduction. 3<sup>rd</sup> Edition. Prentice Hall, Englewood Cliffs, NJ