JOURNAL OF ANIMAL SCIENCE
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J ANIM SCI 2013, 91:3014-3021.
doi: 10.2527/jas.2012-5887 originally published online December 10, 2012

TRIENNIAL REPRODUCTION SYMPOSIUM: Influence of follicular characteristics at ovulation on early embryonic survival[1,2]

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ABSTRACT: Reproductive failure in livestock can result from failure to fertilize the oocyte or embryonic loss during gestation. Although fertilization failure occurs, embryonic mortality represents a greater contribution to reproductive failure. Reproductive success varies among species and production goals but is measured as a binomial trait (i.e., pregnancy), derived by the success or failure of multiple biological steps. This review focuses primarily on follicular characteristics affecting oocyte quality, fertilization, and embryonic health that lead to pregnancy establishment in beef cattle. When estrous cycles are manipulated with assisted reproductive technologies and ovulation is induced, duration of proestrus (i.e., interval from induced luteolysis to induced ovulation), ovulatory follicle growth rate, and ovulatory follicle size are factors that affect the maturation of the follicle and oocyte at induced ovulation. The most critical maturational component of the ovulatory follicle is the production of sufficient estradiol to prepare follicular cells for luteinization and progesterone synthesis and prepare the uterus for pregnancy. The exact roles of estradiol in oocyte maturation remain unclear, but cows that have lesser serum concentrations of estradiol have decreased fertilization rates and decreased embryo survival on d 7 after induced ovulation. When length of proestrus is held constant, perhaps the most practical follicular measure of fertility is ovulatory follicle size because it is an easily measured attribute of the follicle that is highly associated with its ability to produce estradiol.

Key words: cattle, embryonic survival, ovary, pregnancy

 J. Anim. Sci. 2013.91:3014–3021
doi:10.2527/jas2012-5887

INTRODUCTION

Reproductive success is of critical importance to production efficiency for all domestic livestock species. Although measures of reproductive success may vary among species and production goals, it is ultimately a binomial trait (i.e., pregnancy), derived by the success or failure of multiple biological steps. Understanding these biological steps allows for progress to be made towards alleviating reproductive failures, but progress toward this goal is limited. The inability to repeatedly determine pregnancy success during early gestation and before conceptus implantation prevents the complete characterization of the individual steps involved in early embryonic survival. Consequently, pregnancy establishment is often used to indicate success or failure of the trait of interest. Ultimately, pregnancy success is the summation of such simplistic steps as ovulation of a viable oocyte, sperm transport and fertilization, regular cell division and growth by the developing embryo, secretion and reception of pregnancy recognition signals that ultimately prevent

[1]Based on a presentation at the Triennial Reproduction Symposium titled "Impediments to Fertility in Domestic Animals" preceding the Joint Annual Meeting, July 15–19, 2012, Phoenix, Arizona. The symposium was sponsored, in part, by the The James Lauderdale Appreciation Club, The ASAS Foundation, Elanco Animal Health (Greenfield, IN), and Pfizer Animal Health (New York, NY), with publication sponsored by the American Society of Animal Science and the *Journal of Animal Science*.

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Received September 21, 2012.
Accepted November 19, 2012.

luteolysis, and placentation within the uterus. The symposium for which this paper is written attempted to break down each of these steps and focus on the most critical characteristics affecting reproductive success.

It is logical to assume that the ovarian factors affecting reproductive success are optimized when a female ovulates spontaneously after a natural estrus and is inseminated by a fertile male. With the advent of assisted reproductive technologies to manipulate reproduction, optimization of each component required for reproductive success is not always achieved. The objective of this review is to explore the roles of ovarian follicular characteristics that are involved in early embryonic survival. The majority of this review is focused on beef cattle, in which assessment of reproductive success (i.e., pregnancy establishment) is difficult before d 27 of gestation.

OVARIAN CHARACTERISTICS AFFECTING PREGNANCY ESTABLISHMENT

Reviews have been published on follicular determinants associated with pregnancy (Mermillod et al., 1999; Pohler et al., 2012); however, few of these determinants involve characteristics that can be measured in vivo. As the female of any species approaches ovulation, the ovulatory follicle undergoes maturational changes that are associated with increasing follicular size and alterations in hormone production. These maturational steps are controlled by endocrine, paracrine, and autocrine signals that not only prepare the oocyte for fertilization but also signal the entire reproductive tract to prepare for pregnancy. In cattle, application of assisted reproductive technologies manipulates the normal sequential events of follicular growth and the estrous cycle to synchronize follicular development, initiate luteolysis, induce ovulation of a fully mature follicle, and mimic hormonal profiles associated with estrus and normal estrous cycles. In many instances, estrous cyclicity may be induced in cows that have not resumed cycling after parturition or heifers that are prepubertal. Therefore, the question associated with this review becomes "how do we manipulate the estrous cycle in a manner to ensure the ovulation of an oocyte from a follicle that has received and relayed the appropriate stimulation to result in a successful pregnancy rather than ovulation of an oocyte from a follicle that is deficient in its ability to become fertilized or develop into an embryo capable of surviving?" Some characteristics that may affect this response include optimizing ovulatory follicle size (Lamb et al., 2001; Vasconcelos et al., 2001; Perry et al., 2005), duration of proestrus (Taponen et al., 1999; Peters and Pursley, 2003; Bridges et al., 2008, 2010), and duration of ovulatory follicle dominance (Sirois and Fortune, 1990; Mihm et al., 1994; Cerri et al., 2009). Additional variables that may assist in creation of the optimal ovulatory follicle include follicular growth pattern (Townson et al., 2002; Bleach et al., 2004), follicle age (Abreu et al., 2011 a; 2011b), and steroid microenvironment (Fortune et al., 2004) in which final follicular development occurs.

FOLLICULAR DETERMINANTS OF OOCYTE COMPETENCE

Folliculogenesis, oocyte growth, and oocyte maturation depend upon bidirectional communication between the oocyte and surrounding follicular cells (Eppig, 2001; Matzuk et al., 2002). In mammals, acquisition of oocyte competence is required for embryonic development and survival (Krisher, 2004). Sirard et al. (2006) defined oocyte competence as the ability of an oocyte to resume meiosis after gonadotropin stimulation, undergo cleavage divisions after fertilization, develop to the blastocyst-stage embryo, and result in birth of live offspring. Although it is safe to expect that only competent oocytes would successfully achieve each of these steps, there are many additional reasons why pregnancies derived from competent oocytes may fail.

Follicular diameter has been positively associated with acquisition of oocyte competence in several species. Oocytes recovered from large follicles had improved developmental competence in sheep (Moor and Trounson, 1977), pigs (Ito et al., 2008), horses (Goudet et al., 1997), and cattle (Mermillod et al., 1999; Sirard et al., 2006) compared with oocytes from smaller follicles. At the secondary stage of follicle growth in cattle, the zona pellucida begins to form adjacent to the vitelline membrane, cortical granules begin to form in the oocyte cytoplasm, and RNA synthesis is initiated (Fair et al., 1997a,b). As follicular growth progresses to the tertiary stage, oocyte growth and transcriptional activity increase until the oocyte is about 110 µm in diameter (2 to 3 mm diameter follicle; Crozet et al., 1986; Fair et al., 1995, 1996) at which point bovine oocytes were considered meiotically competent (Fair et al., 1995). Fertilization of oocytes is possible from follicles as small as 2 to 3 mm in diameter, but acquisition of oocyte competence continues in bovine follicles up to 15 mm in diameter as the oocyte continues to acquire mRNA and proteins (Arlotto et al., 1996). Fertilization rate was not greater for larger (>115 µm) versus smaller (<114 µm) bovine oocytes; however, there was an increased rate of morula and blastocyst development after fertilization of the larger oocytes (Arlotto et al., 1996; Pohler et al., 2012).

Bovine oocyte competence continually improves as follicles advance in development, and oocytes from follicles exposed to an LH surge were more competent than oocytes from cows not exposed to an LH surge

(Sirard et al., 2006). Therefore, circulating pulses of LH during the preovulatory period may be beneficial for the final maturation of the oocyte. Sirard et al. (2006) argued that although many oocytes attain meiotic and cytoplasmic competence, the molecular milieu of an oocyte may determine the potential for embryonic and fetal development culminating in birth of viable offspring.

OVULATORY FOLLICLE SIZE

In cattle, ovulatory capacity of a follicle is obtained between 7 and 10 mm in diameter (Sartori et al., 2001; Gimenes et al., 2008) and is associated with acquisition of LH receptors in granulosa cells. However, a greater dose of exogenous LH was required to induce ovulation of a 10-mm follicle versus larger follicles (Sartori et al., 2001). Numerous laboratories have used ovulatory follicle size in cattle as an indicator of physiological maturity at GnRH-induced ovulation (Perry et al., 2005, 2007; Bello et al., 2006; Lopes et al., 2007; Meneghetti et al., 2009; Peres et al., 2009; Sá Filho et al., 2009, 2010). Although this in vivo measurement has been a classical indicator of follicular maturity, the mechanism or mechanisms by which ovulatory follicle size affects fertility have not been elucidated in vitro. Among actively growing follicles, larger follicles contained oocytes with greater nuclear maturation and resulted in greater success with in vitro maturation (**IVM**) and in vitro fertilization (**IVF**) in several livestock species (Moor and Trounson, 1977; Goudet et al., 1997; Mermillod et al., 1999; Ito et al., 2008). In vitro incubation of immature pig oocytes in follicular fluid from large follicles increased nuclear maturation of the oocytes and fertilization rate (Ito et al., 2008). Given the limitations associated with early pregnancy diagnosis in cattle, perhaps the best examples for the use of ovulatory follicle size as a valid indicator of physiological maturity arise from in vivo studies (Lamb et al., 2001; Vasconcelos et al., 2001; Perry et al., 2005, 2007; Lopes et al., 2007).

Lamb et al. (2001) and Vasconcelos et al. (2001) were first to report increased pregnancy rates in cows that had ovulated larger follicles. Lamb et al. (2001), Perry et al. (2005, 2007), and Lopes et al. (2007) demonstrated size thresholds at which pregnancy rates differed between cows and heifers that ovulated large versus small follicles but noted that size thresholds varied between herds (Pohler et al., 2012). Clearly, when cows are treated similarly leading up to induced ovulation, ovulatory follicle size is indicative of reproductive success. However, others have noted that size alone is not a good indicator of fertility when differences exist between ovulation synchronization protocols or when induction of ovulation occurs at different time intervals after induced luteolysis (Mussard et al., 2007; Bridges et al., 2008, 2010). Serum concentrations of estradiol at the time of GnRH-induced ovulation and of progesterone during the next luteal phase were positively correlated to ovulatory follicle size in cows (Perry et al., 2005; Atkins et al., 2008, 2010a,b; Busch et al., 2008). Furthermore, cows induced to ovulate small follicles had reduced serum concentrations of estradiol when compared with cows that spontaneously ovulated either small or large follicles (Vasconcelos et al., 2001; Busch et al., 2008). Speculations are that increased serum estradiol concentration may be related to physiological maturity of the dominant follicle. In addition, ovulatory follicle size did not influence pregnancy rates of cows that ovulated spontaneously after estrus (Perry et al., 2005) even though it was still predictive of fertility in beef heifers (Perry et al., 2007). Induced ovulation of small follicles also increased embryonic mortality between 27 and 60 d of gestation in beef cows (Perry et al., 2005). Some of the factors that influence induced ovulatory follicle size include stage of the estrous cycle at initiation of synchronization and follicular turnover after the first injection of GnRH in synchronization protocols (Atkins et al., 2010a,b). Ovulatory follicle size remains an easily measurable attribute of the follicle that is highly associated with its ability to produce estradiol.

PROESTRUS LENGTH AND OVULATORY FOLLICLE AGE

Increasing the duration of proestrus was positively associated with pregnancy rate after spontaneous (Geary et al., 2010) and induced ovulation (Mussard et al., 2003, 2007; Bridges et al., 2008, 2010) in beef and dairy (Santos et al., 2010) cattle. Bridges et al. (2010) reported increased pregnancy rate was associated with increased length of proestrus after induced ovulation without an accompanying increase in ovulatory follicle size. Increased duration of proestrus was also associated with increased concentrations of serum estradiol at the time of induced ovulation and increased concentrations of serum progesterone during the subsequent luteal phase (Bridges et al., 2008). As highlighted subsequently in this article and in a companion manuscript from this symposium (Bridges et al., 2013), having increased concentrations of estradiol before ovulation and progesterone concentrations after ovulation are requisite for optimal fertility in cattle.

Ovulation synchronization programs that increase duration of proestrus influence the steroid environment in which final follicular growth and maturation occur. Subluteal concentrations of progesterone have been associated with greater follicular development (Savio et al., 1993) through increased LH pulse frequency and dominant follicle maintenance (Roberson et al., 1989;

Sirois and Fortune, 1990; Savio et al., 1993; Wehrman, 1993; Taft et al., 1996). During the luteal phase, circulating progesterone concentrations are high (i.e., 5 to 8 ng/mL), LH pulse frequency is diminished to approximately 1 pulse every 4 h, and the dominant follicle becomes atretic. However, decreased progesterone concentrations (i.e., to 1 to 2 ng/mL) resulted in increased LH pulse frequency (i.e., to 1 or 2 pulses every 2 h), maintenance of the dominant follicle, and increased serum concentrations of estradiol (Savio et al., 1993; Stock and Fortune, 1993; Ahmad et al., 1995).

To increase the duration of proestrus in ovulation synchronization protocols (Bridges et al., 2008; Santos et al., 2010), the interval from follicular emergence to induced luteolysis was reduced, resulting in potential differences in age of the ovulatory follicle that may somewhat confound interpretation of the role of proestrus duration on fertility. Cows exposed to the shorter proestrus period received an injection of GnRH 9.5 d before induced ovulation whereas cows receiving a greater period of proestrus had an 8 d interval from GnRH to induced ovulation. Because the interval from emergence to ovulation or atresia is 7 to 10 d (Ginther et al., 1989), it is likely that more of the cows induced to ovulate 9.5 d after the first GnRH injection had greater variation in age and maturity status of the ovulatory follicle at induced ovulation, in agreement with Atkins et al. (2010b).

It is well recognized that administering subluteal concentrations of progesterone can extend follicle dominance beyond normal lifespan and result in formation of a persistent follicle. Ovulation of a persistent follicle produces a lower quality oocyte (Revah and Butler, 1996) with decreased embryonic development and fertility (Mihm et al., 1994; Ahmad et al., 1995). However, even in the absence of persistent follicles, follicle and oocyte age may contribute to fertility in cattle. This is highlighted by evaluation of the effects of number of follicle waves during an estrous cycle on fertility.

During the estrous cycle, cattle experience 2 or 3 waves of follicular growth (Pierson and Ginther, 1984). After follicular turnover or ovulation, each follicular wave is initiated as a result of FSH stimulation (Sirois and Fortune, 1988). During each follicular wave, a dominant follicle develops as the subordinate follicles undergo atresia (Knopf et al., 1989; Pierson and Ginther 1987; Fortune et al., 1988). The number of follicular waves during the estrous cycle has been related to duration of the luteal phase and the estrous cycle (Ginther et al., 1989; Taylor and Rajamahendran, 1991). The ovulatory follicle in a 3-wave cow emerges later in the estrous cycle in comparison with a 2-wave pattern and thus ovulates at a younger age. Fertility of cows with 3 waves of follicular growth was greater than cows with 2 waves of follicular growth (Townson et al., 2002; Bleach et al., 2004). However, Ahmad et al. (1997) was unable to detect a difference in pregnancy rates to AI (82 and 100%) in beef cows and heifers after spontaneous ovulation of follicles originated either after 2 or 3 waves of follicular development during the estrous cycle, respectively.

Bleach et al. (2004) and Cerri et al. (2009) reported that duration of dominance of the ovulatory follicle impacted embryonic development and pregnancy success in dairy cattle. More specifically, increased duration of follicular dominance led to decreased embryonic quality and pregnancy rate, but it is possible that elevated serum concentrations of progesterone during the majority of follicular dominance affected these results. Abreu et al. (2011a,b) directly compared effects of ovulatory follicle age on fertility in beef cattle and reported that pregnancy rates were not affected by ovulatory follicle age. Abreu et al. (2011a,b) used estradiol benzoate to induce follicular turnover in cows and heifers whose estrous cycles had been synchronized to create a young (approximately 5 d old) or mature (approximately 9 d old) ovulatory follicle at induced luteolysis. Females received AI after spontaneous ovulation in heifers or induced ovulation in cows. Pregnancy rates of heifers (67 and 64%) and cows (67 and 72%) were not different between ovulatory follicle age groups (young and mature, respectively). Therefore, the effects of increased length of proestrus on pregnancy rates observed by Bridges et al. (2008) and Santos et al. (2010) were likely independent of the effects on ovulatory follicle age and more likely a result of increased estradiol and subsequent progesterone on intrafollicular and/or uterine effects.

In cattle, follicular fluid estradiol concentrations before ovulation may affect oocyte maturation and competence (Driancourt et al., 1998; Van de Leemput et al., 1999; Oussaid et al., 1999). Oocytes obtained from bovine preovulatory follicles that had greater intrafollicular concentrations of estradiol were more likely to develop into a blastocyst after IVM, fertilization, and culture (Van de Leemput et al., 1999). Additionally, healthy follicles that had increased aromatase activity (indicating increased estradiol production) and inhibin α subunit were more likely to contain an oocyte capable of developing to the blastocyst stage (Driancourt et al., 1998). In sheep, inhibiting LH pulsatility during the preovulatory period with a GnRH antagonist (Antarelix; American Custom Chemicals Corporation, San Diego, CA), which decreased follicular estradiol production, decreased the proportion of oocytes that were fertilized and the proportion of blastocysts recovered on d 8 of gestation (Oussaid et al., 1999). Given the role of LH in maturation of the oocyte (Gong et al., 1995) and stimulating estradiol secretion by the follicle (Schallenberger et al., 1984), increasing LH secretion during the follicular wave may benefit oocyte competence and improve the chances of conception upon ovulation.

PREOVULATORY ESTRADIOL SECRETION AND PREGNANCY

Estrus occurs after an increase in serum concentrations of estradiol (Allrich, 1994). Preovulatory estradiol coordinates several physiological processes that contribute to the establishment and maintenance of pregnancy, including effects on follicular cells, the oocyte, gamete transport, and preparation of the uterine environment. Within the ovarian follicle, estradiol increases granulosal cell mitosis (Goldenberg et al., 1972), promotes gap junction formation among granulosal cells (Merk et al., 1972), increases the stimulatory action of FSH on aromatase activity (Zhuang et al., 1982), and induces FSH and LH receptor expression in granulosal cells (Richards et al., 1976). Increased concentrations of estradiol within the follicular microenvironment regulate expression of several steroidogenic enzymes (Gore-Langton and Armstrong, 1994) and may impact bovine oocyte maturation and competence directly through genomic estrogen receptors present in the oocyte and/or indirectly through receptors in cumulus cells surrounding the oocyte (Driancourt et al., 1998).

In beef cattle, oocytes from preovulatory follicles with greater concentrations of estradiol were more likely to develop into blastocysts after IVM and IVF (Mermillod et al., 1999). Furthermore, competence of bovine oocytes to be fertilized increased as follicular diameter increased (Arlotto et al., 1996), and increased ovulatory follicle size resulted in greater estradiol production (Martin et al., 1991); however, data examining the specific effects of estradiol on the maturing oocyte are limited. Addition of estradiol to IVM media resulted in either a detrimental effect or no effect on nuclear maturation of bovine oocytes (Beker-van Woudenberg et al., 2004, 2006). Among cattle, serum estradiol concentrations peak approximately 36 h before ovulation (Chenault et al., 1975), and increased preovulatory concentrations of estradiol resulted in increased pregnancy success (Perry et al., 2005, 2007; Lopes et al., 2007; Bridges et al., 2010). Exogenous estradiol administration to cows 24 h before induced ovulation improved pregnancy rates among cows ovulating small (i.e., <12.2 mm diameter) but not large follicles (Jinks et al., 2013). Colazo et al. (2004) also reported increased pregnancy rates in beef heifers receiving estradiol before AI. The ability of exogenous estradiol to have a major effect within the ovulatory follicle seems unlikely given the increased concentrations of estradiol in follicular fluid and that healthy ovulatory follicles are not vascularized within the membrane granulosa (Rodgers and Irving-Rodgers, 2010). Therefore, preovulatory concentrations of estradiol likely have the greatest impact on pregnancy success through gamete transport or regulation of the uterine environment.

POSTOVULATORY CONCENTRATIONS OF PROGESTERONE

The corpus luteum is the primary source of progesterone during the establishment of pregnancy in cattle. The corpus luteum is a continuation of follicular maturation, and preparation of follicular cells to luteinize, synthesize, and secrete progesterone is initiated before ovulation. Inadequate gonadotropin secretion and/or decreased estradiol production during the preovulatory period may have adverse effects on subsequent luteal lifespan or progesterone secretion. Progesterone is known to maintain pregnancy through promoting uterine functionality and the uterine microenvironment (see Bridges et al., 2013) but follicular characteristics are known to affect subsequent progesterone concentration in cattle (Perry et al., 2005, 2007; Mussard et al., 2007; Busch et al., 2008; Atkins et al., 2010a,b; Bridges et al., 2010). A recent review by Fair and Lonergan (2012) describes the effects of circulating concentrations of progesterone on oocyte developmental competence.

RECIPROCAL EMBRYO TRANSFER: OVARIAN CHARACTERISTICS ASSOCIATED WITH PREGNANCY

A recently conducted reciprocal embryo transfer experiment differentiated between follicular effects on oocyte quality and the uterine environment on the establishment and maintenance of pregnancy in cattle (n = 1,164) among which ovulation was induced with GnRH (d 0). Atkins et al. (2013) reported that both reduced oocyte competence and a compromised uterine environment contributed to decreased pregnancy rates of cows induced to ovulate small dominant follicles. The study by Atkins et al. (2013) used path analyses (Wright 1920, 1934) to delineate between direct and indirect effects of multiple variables on several measures of fertility. In this study, 90% of the follicular structures recovered at embryo transfer on d 7 were fertilized embryos, which is in agreement with results of others (Maurer and Chenault, 1983; Ahmad et al., 1995). All embryos that were determined to be viable (93%) were transferred into recipients regardless of embryonic developmental stage or quality. The probability of fertilization and recovering a transferable embryo were both positively associated with follicle size. Consequently, the follicular microenvironment and its effect on oocyte competence appears to have critical roles in subsequent pregnancy

Table 1. Ovarian variables of embryo donor cows having the greatest effect on measures of reproductive success (fertilization, embryo viability, embryo developmental stage, and embryo quality) measured on d 7 in beef cows[1]

Reproductive measure	Effect
Fertilization	
1. Day 0 serum estradiol concentration	Positive
2. Ovulatory follicle size	Positive
3. Ovulatory follicle growth rate	Positive
Embryo viability	
1. Day 7 serum progesterone concentration	Positive
2. Day –2 serum progesterone concentration	Positive
3. Day –9 follicular turnover	Positive
4. Ovulatory follicle size	Positive
5. Day 0 serum estradiol concentration	Positive
Embryo stage	
1. Day –2 serum progesterone concentration	Positive
2. Day 7 serum progesterone concentration	Positive
Embryo quality	
1. Ovulatory follicle size	Positive
2. Day 0 serum estradiol concentration	Positive
3. Day 7 serum progesterone concentration	Positive

[1]Adapted from Atkins et al. (2013).

establishment and maintenance. Variables having the greatest effect on fertilization success were increased serum concentrations of estradiol at induced ovulation, increased ovulatory follicle size, and increased growth rate during the 48 h preceding induced ovulation. The variables that had the greatest influence on recovery of a live embryo on d 7 after induced ovulation were increased serum concentration of progesterone on d 7 and –2, follicular turnover as a result of GnRH administration on d –9, increased ovulatory follicle size, and increased serum estradiol (Table 1). Increased serum concentration of progesterone on d –2 and 7 had the greatest positive influence on embryonic developmental stage on d 7 whereas increased ovulatory follicle size, d 0 estradiol concentration, and d 7 progesterone concentration were the primary variables influencing recovery of a better quality embryo. Increased ovulatory follicle size was the only donor cow variable that improved pregnancy retention from d 28 to d 72 (Atkins et al., 2013). Because pregnancy establishment in embryo recipient cows (assessed on d 28) was affected only by recipient cow traits, the primary limitation to pregnancy establishment, once a fertilized embryo has reached the uterus, is likely at the level of the uterus (Atkins et al., 2013; Bridges et al., 2013).

SUMMARY AND CONCLUSIONS

In summary, there are multiple steps associated with the ovulatory follicle that affect oocyte growth, fertilization, embryonic development, and establishment of pregnancy. When estrous cycles are manipulated with assisted reproductive technologies and ovulation is induced, some of these variables become more important. Duration of proestrus (i.e., the interval from induced luteolysis to induced ovulation), ovulatory follicle growth rate, and ovulatory follicle size are factors that affect the maturation of the follicle and oocyte at induced ovulation. Perhaps the critical maturational component is the production of sufficient estradiol by the ovulatory follicle to prepare follicular cells for luteinization and progesterone synthesis needed to prepare the uterus for pregnancy. The exact roles of estradiol in oocyte maturation remain unclear, but cows that had lesser serum concentrations of estradiol had decreased fertilization rates and decreased embryonic survival on d 7 after induced ovulation. When duration of proestrus is held constant, perhaps the most practical follicular measure of fertility is ovulatory follicle size because it is an easily measured attribute of the follicle that is highly associated with its ability to produce estradiol.

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