MINIREVIEW

Embryogenesis Recapitulates Oogenesis in Swine (43356B)

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Abstract. **Events during oogenesis can affect embryogenesis so dramatically that oocytes can be identified that are progenitors of embryos which would probably die if they remained in the host pig, but would live if appropriately transferred to another female. This review goes backward from embryonic to oocyte development, first discussing how subtle differences between littermate embryos can result in the death of some embryos and then relating the causes of those differences to events during follicular maturation. Embryonic development is not uniform in swine. The larger blastocysts within a litter synthesize estradiol sooner than their less developed contemporaries. Estradiol advances uterine secretions to the benefit of the more developed blastocysts, but results in an asynchronous and hostile environment for the less developed blastocysts. Through a series of experiments, the pattern of oocyte and follicular development was found to be one of the sources of subsequent disparity among blastocysts. In pigs mated before ovulation, the first oocytes released at ovulation were the first fertilized and became the more developed blastocysts 12 days later. Inversely, the later ovulated oocytes were the last to be fertilized and became the smaller blastocysts. These smaller blastocysts can develop normally, but because of estrogenic advancement of uterine secretions, they will preferentially die.** [P.S.E.B.M. 1992, Vol 199]

The death of initially viable embryos is one of the major limitations to improving reproductive performance and little size in swine. Fertilization rates (the percentage of oocytes fertilized of those ovulated) are typically greater than 95%, and surveying the number of corpora lutea and concepti recovered from a large number of animals has allowed assessment of the proportion of embryos dying (embryonic mortality). From several studies it has been accepted that during the first 25 to 30 days of gestation, embryonic mortality is approximately 30% (1–12). Recent advances in the understanding of embryonic development and uterine physiology have allowed the introduction of a possible mechanism of embryonic mortality in swine.

This review will consider how subtle differences among littermate concepti result in the loss of some blastocysts but the survival of others. These differences in embryogenesis will be related to events during oogenesis, ovulation, and fertilization. Finally, a review of possible methods to alter oogenesis and embryogenesis will be considered in an attempt to reduce embryonic mortality.

Hypothetical Model of Embryonic Mortality

Development among littermate concepti is not uniform in swine. Blastocysts hatch from the zona pellucida on the seventh day after the onset of estrus, designated as Day 7 (Day 0, onset of estrus; Day 1, ovulation). Hatched blastocysts grow in a spherical morphology for 4 days and then begin to elongate into ovoidal, tubular, and filamentous shapes (13). During the transition from spherical to filamentous morphology, the variability (coefficient of variation for diameter) among blastocysts is most apparent (14). It is normal for recovered concepti on Day 12 to range morphologically from 5- to 6-mm spherical to 10- to 20-cm filamentous blastocysts. Geisert *et al.* (15) hypothesized that cellular reorientation and remodeling along the long axis of the blastocyst accounted for most of the rapid growth (up to 3–4 cm/hr) during the

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transition from the spherical to filamentous morphology.

It is difficult to assess how long a large spherical blastocyst takes to become filamentous. Utilizing a model of partial hysterectomy before the transition, followed by a second surgery to remove the remaining gravid uterine tissue during or after the transition to filamentous morphology, allows estimating an interval of 2–3 hr (R. D. Geisert, personal communications).

The disparity among littermate blastocysts by Day 12 is functional as well as morphological. At this time, porcine blastocysts are secreting polypeptides (16), plasminogen activator (17), estradiol (18), catecholestradiol (19), prostaglandins $F_2\alpha$ and E_2 (20), and interferons (21). For example, within a litter, the filamentous embryos synthesize, step wise, more estradiol than the tubular, the tubular more than the ovoidal, and the ovoidal more than the spherical blastocysts (20, 22, 23).

On Days 11 and 12, the progesterone-primed endometrium is very sensitive to estradiol and will alter stromal and epithelial development (24) and secretions (25–27) in response to embryonic estrogens. Geisert *et al.* (25) injected estradiol valerate on Day 10, instead of the normal endogenous exposure to estradiol on Day 11, and observed earlier release of calcium ions and specific proteins into the uterine lumen. This earlier release was considered an effect of estradiol to signal advancement in uterine function. Initially, these uterine responses are compartmentalized into microenvironments within an area of the lumen that contains a blastocyst of a specific morphology (28). Eventually, as the more developed blastocysts secrete more estradiol, larger and larger portions of the uterus become exposed and the distinction among the compartments probably diminishes.

Porcine blastocysts remain free-floating in the uterus until the initial stages of attachment begin on Day 13 (29). During this comparatively long period of being unattached within the lumen, all the nutritive requirements for survival have to be supplied by the uterine milieu (historically considered "hystotroph" or uterine milk). Results of experiments comparing media- to culture-hatched blastocysts suggest that survival of advancing stages of embryonic development requires increasingly complex secretions from the uterus (30). Alternatively, blastocysts transferred to an advanced environment (a recipient in estrus before the donor) will not survive as well as those transferred synchronously (11, 31, 32). These observations suggest that an advancing uterine environment can be beneficial and complementary to the advancing requirements for normal blastocyst growth, but exposing a blastocyst to an environment that is too asynchronous could be detrimental. In support of this concept, when estradiol advances uterine secretions too early in gestation, then partial or complete embryonic loss occurs (26, 27, 33–35).

Under natural conditions, concepti are randomly mixed within the uterus with smaller spherical or ovoidal blastocysts situated adjacent to larger tubular and filamentous blastocysts (36). Within a litter, the sensitivity of the smaller versus larger blastocyst to uterine advancement is also evident. Wilde *et al.* (37) observed that the less developed (smaller diameter) Day 7 blastocysts, after being transferred to Day 7 recipients, were less likely to survive compared with the more developed littermates. However, these less developed blastocysts could survive similarly to the more developed blastocysts if they were transferred to Day 6 (less advanced) recipients. Pope *et al.* (38) proposed that as the more developed blastocyst in the litter begins to synthesize estradiol, the uterine milieu is advanced first in a local manner and to the benefit of those blastocysts. As the amount of estradiol exposure increases, so does the degree of advancement, and this stimulation encroaches to segments of the uterus containing the less developed blastocysts of the litter. These smaller blastocysts become compromised and may be lost.

Several lines of investigation support the hypothetical model of the larger blastocysts, through their earlier synthesis of estradiol, advancing uterine secretions to the detriment of the smaller blastocysts. First, Xie *et al.* (28) ligated the bicornuate uterus of porcine recipients at the external bifurcation and then transferred to one side the smaller blastocysts and to the other side the larger blastocysts of a litter. On Day 12, the uterine horn containing the larger littermate blastocysts was associated with a uterine environment that contained more estradiol, acid phosphatase, total protein, and, from two-dimensional polyacrylamide gel electrophoresis procedures, secretory proteins indicative of a more advanced endometrium function (28). Second, transferring genetically tagged Day 5 and 7 embryos into nonpregnant Day 6 recipients indicated no difference in the ability of these embryos to survive the transfer procedures (Day 11), but by the time coat color was established on Day 60, clearly those embryos that were more developed early in gestation had a survival advantage (39; Table I). Comparing the ability of Day 5 embryos to survive in Day 6 recipients to Day 60 without the presence of the larger, transferred-Day 7 embryos indicated a similar survival rate to the transferred-Day 7 embryos (42% vs 63%, respectively). Collectively, these data suggest similar abilities of the Day 5 and Day 7 embryos to survive, but, when forced to cohabit the uterus, the more developed embryos predominated. Finally, by increasing the proportion of embryos within a litter that are further developed, the total percentage of embryos surviving to Day 30 also increases (40).

Table I. Percentage Survival of Day-5 and -7 Pig Embryos to Days 11 and 60 of Gestation after Transfer to Day-6 Recipients[a]

	No. of recipients utilized	No. of recipients pregnant	Day-5 embryos			Day-7 embryos		
			No. of embryos transferred	No. surviving	Survival/ recipient (%)	No. of embryos transferred	No. surviving	Survival/ recipient (%)
Day 11	10	8	45	19	42.3 ± 10.4*	42	18	43.1 ± 12.4*
Day 60	16	8	87	6	8.2 ± 6.9†	83	53	62.6 ± 7.7‡
Day 60	10	5	88	35	42.0 ± 9.8			

[a] From Pope *et al.* (39). Means with different superscripts (*, †, ‡) within rows are different ($P < 0.001$).

Table II. Developmental Stages of Zygotes[a]

Gilt	Stage of maturation			
	Met II	Ana II/Telo II	PN	PNMF
1	5	5		
2	5	11		
3	2		7	2
4	3		7	
5		2	6	
6		2	5	4
7		4	4	4
8		3	8	
9		1	3	10
10		3	5	5
11		2	10	2
12		3		11
13			4	15
14			7	
15			7	1
16			2	6
17			3	9
18			3	10
19			3	11

[a] Developmental stages of zygotes collected from gilts 5–7 hr after initiation of ovulation (from Xie *et al.* [54]). Each row represents zygotes recovered from one gilt. Zygotes were collected 39–42 hr after the onset of estrus. Met, Metaphase; Ana, Anaphase; Telo, Telophase; PN, pronuclear; PNMF, pronuclear membrane fusion.

Sources of Embryonic Disparity

Within a litter, variation in blastocyst development on Day 12 is not due to preferential sites within the uterus, breed of the male or female, or the sequence of embryos hatching from the zona pellucida. Late in gestation, fetuses grow faster in some areas of the uterus than in others (6, 41, 42); however, blastocyst development (13) and uterine blood flow (43) are initially similar among segments of the gravid uterus. Migration of blastocysts through the uterine lumen is unrelated to the degree of disparity among embryos. Blastocysts begin to migrate on Day 7 and cease further movements by Day 12 (44, 45). Embryonic estradiol appears to be one of the signals that initiates migration (46), and although the more developed embryos synthesize estradiol before the less developed embryos, the uterus lacks the ability to discriminate among embryos and blastocysts become intermixed between the left and right sides (47, 48). Similarly, the genotype of the litter does not appear to effect disparity among embryos. Differences have been observed in the rate of embryonic growth between domestic, miniature, and Chinese breeds of swine (49, 50); however, each breed has equivalent amounts of disparity among embryos on a within-litter basis. Finally, the range of blastocyst development on Day 12 is also unrelated to the process of hatching. Broermann *et al.* (51) enzymatically induced premature hatching from zonae and failed to minimize the extremes or degree of disparity among littermate blastocysts.

The search for sources of differences among littermate embryos extended back before the preblastocyst stage. Because it was common to recover embryos ranging developmentally across several stages of early

Table III. Variability in Hormone Content of Follicles ($n = 453$)[a]

Measure of variability	P	E	A	T	DS	$PGF_{2\alpha}$	PGE_2
r	0.64[b]	−0.81[b]	−0.69[b]	−0.56[b]	0.38[b]	0.33[b]	0.38[b]
Coefficient of skewness[c]	−1.30[d]	0.95[d]	0.89	0.83	1.15[d]	−1.19[d]	−1.31[d]

[a] Hormones were correlated (r) with stage of oocyte maturation and examined as coefficients of skewness (from Xie *et al.* [58]). P, progesterone; E, estradiol; A, androstenedione; T, testosterone; DS, dermatan sulfate; PGE_2 and $F_{2\alpha}$, prostaglandin E_2 and $F_{2\alpha}$.
[b] $P < 0.01$.
[c] Mean coefficient was determined for the distribution of each hormone within each gilt.
[d] $P < 0.05$.

Table IV. Number and Percentage of Atretic Follicles during Oocyte Maturation[a]

	Total number of follicles	Atretic follicles[b]	
		(*n*)	(%)
Stage			
GV	68	14	20.5*
GVBD	21	4	19.0*
Met I	125	16	12.8*
Ana I/Telo I	58	2	3.4†
Met II	181	6	3.3†
Hour			
21	96 (6)[c]	13	13.5
24	90 (5)	8	8.9
27	92 (5)	8	8.7
30	88 (5)	8	9.0
34	87 (5)	5	5.7

[a] From Xie *et al.* (58). GV, *germinal vesicle*; GVBD, *germinal vesicle* breakdown; Met, Metaphase; Ana, Anaphase; Telo, Telophase.
[b] Percentage indicates total number of atretic follicles/total number of follicles examined. Means with different superscripts (*, †) differ ($P < 0.05$).
[c] The number in parentheses indicates the number of gilts examined. The ovulation rate for contemporary herdmates is 14–17.

cleavage, it was of interest to examine whether differences among blastocysts existed at the zygotic or oocyte stages. Second, if oocyte development was not uniform and synchronous, then experiments could be conducted to consider whether developmental differences continued through ovulation and fertilization. Finally, one could examine whether differences that existed in gametogenesis were directly related to differences in embryonic development 12 days later.

Gilts begin ovulating approximately 34 hr after the onset of estrus (47, 52, 53). Xie *et al.* (54) collected zygotes 5–7 hr after the initiation of ovulation. Recovered zygotes were cytogenetically classified into stages of maturation and were observed to range, within a gilt, across several stages of development (Table II). In one example, Gilt 3 concepti ranged from those in Metaphase II (Met II) to those fusing their pronuclear membranes. Other recovered zygotes frequently ranged within gilts from Anaphase/Telophase II or pronuclear to pronuclear membrane fusion. The distribution of zygotes across these cytogenetic stages was not normal, but was skewed, with the majority of zygotes being further developed than the others.

Continuing backward physiologically, it was of interest to examine whether the pattern of ovulation was related to zygotic diversity. The duration of ovulation has been estimated to occur from 3 to 6 hr (55–57). In an attempt to describe the pattern of ovulation from the first to the last follicle, Pope *et al.* (53) surgically observed the ovaries of 58 gilts at 34 hr after the onset of estrus. The population of visible follicles at this time is either small (2–3 mm) nonovular or large (>6 mm) preovulatory follicles. The percentage of large atretic follicles is only 3% at this time (58), so most of the large follicles can accurately be considered preovulatory. Of the 58 gilts, 18 had not begun to ovulate (no corpora hemorrhagica [CH] present), 25 were in the process of ovulating (both large follicles and CH), and 14 were finished ovulating (only CH and small follicles). Considering the 25 gilts in the process of ovulating, more closely, one gilt was 6% completed with ovulation (one CH and 17 large follicles) and 24 were between 70% and 96% completed with ovulation. The infre-

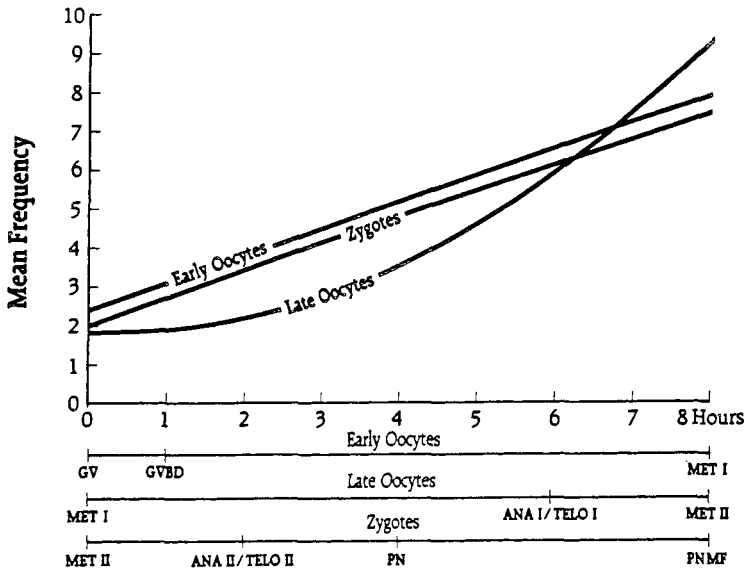

Figure 1. Stages of oocyte maturation and zygote development. Regression of the mean frequency of oocytes and zygotes collected from within a gilt with the estimated duration of stages of early (21–27 hr) and late (30–34 hr) oocyte maturation and zygotic (42 hr) development. Gilts with oocytes classified in germinal vesicle arrest were not included in the early oocyte regression (from Xie *et al.* [54]).

Table V. Subsequent Development of Normal and Aspirated/Stained Oocytes at Various Times after Oviductal Transfer Procedures[a]

Hours after surgery[b]	Stage of embryonic development[c]		
	2 cell	4 cell	8 cell
20	9 (2)		
40	12 (1)	(3)	
60	2	12 (4)	
80		4	(3)

[a] The numbers in parentheses indicate the numbers of aspirated/stained oocytes. From Xie *et al.* (54).
[b] Each row represents embryos recovered from one gilt.
[c] Number of embryos recovered at each stage of development.

Table VI. Distribution of Embryos of Stained or Unstained Oocytes[a]

Time of aspiration	Gilt (*n*)	Ovulation[b] (%)	Stage of recovered embryo					
			4 cell		8 cell[c]		16 cell[d]	
			(S)	(U)	(S)	(U)	(S)	(U)
Before ovulation (39 hr post-hCG)	1	0		4	3			
	2	0		3		8	4	
	3	0		1		3	4	8
	4	0		2		6	3	
	5	0		8	4	3		
	6	0		4	3	3		
Total			0	22	10	23	11	8
During ovulation (42 hr post-hCG)	7	70	4	3		5		
	8	57	4	3		4		
	9	67	2		2	4		3
	10	67	2	2	1	5		
	11	69	2	2	1	9		
	12	71	4	3				
Total			18	13	4	27	0	3

[a] Each row represents the number and classification of embryos recovered from individual gilts at 113–115 hr after hCG. Stained embryos recovered from gilts in the 39-hr group were more ($P < 0.01$) developed than stained embryos of the 42-hr group. S, stained; U, unstained. From Xie *et al.* (28).
[b] Ovulation percent indicates the number of corpora hemorraghica/number of CH + number of large follicles at the time of surgery.
[c] The 8-cells stage includes 6- to 8-cells embryos.
[d] The 16-cell stage includes 12- to 16-cell embryos.

quency of observing gilts from 1% to 70% completion of ovulation compared to the frequency of observing gilts from 70% or more completion of ovulation suggested that ovulation of the first 70% of the follicles was rapid and the remaining minority of follicles ovulated over a longer period of time (53). Statistically, this pattern of ovulation is skewed and similar to the pattern of zygotic development.

What is the pattern of oocyte and follicular development? Changes in steroid concentrations of antral fluid of large follicles of gilts and sows have been well characterized at various hours after the onset of estrus or hours after human chorionic gonadotrophin (hCG) injection (59–61). However, given the possible variability within an animal, Xie *et al.* (58) utilized the cytogenetic stage of oocyte maturation to further classify follicular fluid collected from gilts from 24 to 34 hr after the onset of estrus. Cross-classifying changes in hormonal concentrations of antral fluid with stage of oocyte maturation reduced the residual error in the analysis of variance and resulted in a significant change in all hormones examined at these times. Furthermore, all hormonal changes in the antrum were correlated ($P < 0.01$) with stages of oocyte maturation (Table III). Cross-classifying follicles by stage of meiosis of the oocyte was also more accurate than simply considering hour after the onset of estrus (Table IV) in examining the amount of follicular atresia (62–66) as ovulation approached. The pattern of oocyte and follicular maturation was, therefore, considered to be highly interrelated. In support, oocytes of less than 0.2% of the ovulations of mature females were observed at an immature (pre-Met II) stage of development (67). Again, the distribution of oocyte and follicular development was not normal but skewed (Table III), similar to the pattern of ovulation and zygotic development.

Utilizing estimates of the duration of various stages of meiotic development and early penetration (68–72) allowed us to plot the number of oocytes or zygotes recovered from within a gilt at these various stages into frequency distribution curves for early (up to Met I) and late (up to Met II) oocytes and early zygotes (Fig. 1). Because oocytes accumulate at the Metaphase II stage shortly before ovulation, the late oocytes curve was quadratic. However, the regressed curves for early oocytes and zygotes were linear and of similar slopes (0.59 and 0.58, respectively). The apparent parallelism of these latter curves suggested that zygotic disparity was caused by oocyte disparity, if one assumes the amount of time to fertilize each oocyte was constant.

Standard management practice is to mate gilts and sows before they begin ovulation. Studies using large numbers of animals have shown that conception rates are highest if animals are mated around 6–8 hr before ovulation begins. Utilizing a model of gilts mated 6 hr before ovulation, it was of interest to examine whether the time from ovulation to fertilization was, in fact, constant among all ovulated oocytes. To examine whether the time of fertilization was similar, Xie *et al.* (54) utilized a system of aspirating a subset of the preovulatory follicles shortly before the predicted time of ovulation that were from gilts that were mated 6 hr before surgery. Recovered oocytes were enzymatically stripped of cumulus cells, stained, and transferred back to the oviducts of the same gilts. This was a modified gamete intrafallopian transfer (GIFT) procedure, since the nonaspirated follicles could continue to ovulate their oocytes and the resulting embryos would then be

Table VII. Distribution of Day 12 Blastocysts after Transfer of Littermate, Younger or Older, Day-4 Embryos into Separate Uterine Horns[a]

Recipient	No. of older or younger embryos transferred	Morphology of recovered embryos									
		Spherical				Ovoidal		Tubular		Filamentous	
		1–5 mm		6–10 mm							
		Y	O	Y	O	Y	O	Y	O	Y	O
1	5	2				2			4		1
2	4	2		2	2		1		1		
3	3	2			1		1				
4	4			2			1				3
5	4			2			1		1		
6	4			3		1	3				
7	5							2		2	3
8	5					1		2			5
9	4					1		1			1

[a] Each row represents the number and classification of embryos recovered from each gilt. Younger (Y) Day-4 embryos were lesser ($P < 0.01$) developed blastocysts by Day 12 than older (O) Day-4 embryos by Day 12.

unstained. The stain used on the aspirated oocytes was a fluorescently tagged monobromobimane that bound to thiol moieties in the ooplasm. To verify that such a procedure resulted in normal fertilization and subsequent normal embryonic development, embryos of gilts treated by this modified GIFT procedure were examined at various times after completion of ovulation (Table V). These data indicated two things: the embryos of stained oocytes were slightly more developed, and this relationship continued to the 4- to 8-cell stages. The initial advantage in development of the aspirated oocyte is probably due to physically ovulating/transferring these oocytes to the oviduct earlier than the normal time of ovulation and to an environment void of a follicular milieu that might delay maturation.

Xie *et al.* (54) continued after these preliminary trials by comparing the time of fertilization of oocytes from early maturing follicles to the time of those released after ovulation had begun. If the order of ovulation paralleled the order of embryonic development, then the premise of a constant time to fertilization could be supported. Procedurally, four to five oocytes were aspirated, stained, and transferred to the oviduct of gilts at 39 or 42 hr after hCG injection (500 IU). Proestrus gilts begin ovulating 40–41 hr after hCG; therefore, the 39 or 42 hr post-hCG injection times represented gilts just before or during ovulation, respectively. Recovered embryos were examined 113–115 hr post-hCG at the 4- to 16-cell stage. GIFT (stained) embryos resulting from oocytes randomly aspirated just before ovulation, as before in the preliminary trial, were slightly more developed, whereas the last oocytes to be released, those aspirated from gilts 70% completed with ovulation, were less developed (Table VI). These observations supported the concept that in gilts mated before ovulation, the time to fertilize each oocyte is constant.

Results of the last experiment also demonstrated that the first ovulated oocytes (Table VI; 42 hr, unstained) were at the 8- to 16-cell stage of development before the later ovulated oocytes (Table VI; 42 hr, stained). To examine whether this ordered pattern of development continued to Day 12, late 4-cell embryos were observed frequently during a short incubation period during cleavage to the 8-cell stage. As each embryo began to cleave, it was "picked off" and transferred to another dish. This allowed identification of the older, intermediate, and younger 8-cell embryos. An equal number of the older and younger embryos from a gilt were transferred to separate and ligated uterine horns of nonpregnant recipients. Day 12 blastocysts were subsequently recovered from each horn and, indeed, the more developed blastocysts were those that were older 8-cell embryos at the time of transfer; the smaller blastocysts on Day 12 were from younger 8-cell embryos (Table VII). In a related experiment, Pope *et al.* (53) cauterized the slower developing follicles of mated gilts and observed the absence of small blastocysts normally present on Day 12. Carrying this model to its fruition, the flushings of the uterine lumen containing the more developed Day 12 embryos contained more estradiol, acid phosphatase, and, from two-dimensional polyacrylamide gel electrophoresis analysis, advanced secretions than did the opposite side.

How Can Embryonic Disparity Be Reduced?

To reduce embryonic mortality, it would be of interest to develop a method to reduce the amount of variability among littermate embryos or to reduce the effects of that range in development. Starting with the latter first, attempts to antagonize the uterine advancing effects of estradiol by inhibiting the rate of estradiol synthesis have thus far been difficult. O'Neill *et al.* (73) inhibited the rate of embryonic synthesis of estradiol

by approximately 90% and still observed the normal advancement of uterine secretions on Day 12.

Reducing the disparity among embryos may be a more productive approach. Ford *et al.* (49) observed genetic differences in the rate of embryonic development among haplotypes of miniature swine. Similarly, this laboratory at Iowa State University has observed that in the Chinese Meishan breed, the embryos grow slowly and, perhaps more significant, also have a more gradual secretion and release of estradiol than the more domestic breeds (S. P. Ford, personal communication). Alternatively, hCG can be used to induce ovulation; however, hCG does not alter the skewed pattern of ovulation (53, 74). Perhaps other hormonal therapies to coordinate events during selection and development of preovulatory follicles will become available in the future.

Preliminary results from our laboratory indicate that fertilization after ovulation failed to reduce the amount of disparity among littermate blastocysts. Again utilizing hCG to time the initiation of ovulation, gilts were mated 6–8 hr before versus 2–3 hr after ovulation had begun. At 288 hr after hCG, blastocysts of the delayed fertilization group were smaller than the preovulatory mating (control) group. At a more developmentally comparable time in the delay-mated group, 295 hr, the range of development was similar to that of the control group (H. Cardenas and W. F. Pope, unpublished data). These data would suggest that although sperm penetration initiates embryonic development, more synchronous fertilization of all the oocytes in delay-mated gilts still results in expression of inherent differences among oocytes during maturation, and that these differences continue to the blastocyst stage of development.

Summary

On Days 14 to 16 of the estrous cycle, corpora lutea begin to regress and a pool of medium-sized (3–5 mm) follicles begin to be exposed to increasing concentrations of gonadotropins. Selection and recruitment of follicles from the medium-sized pool does not continue much after Day 18, since few medium-sized follicles are replaced, nor are many medium follicles present by Day 21 (75–77). Grant *et al.* (78) suggested that medium-sized follicles do not respond synchronously to the sequence of events that induce follicular recruitment and selection. Perhaps during the period of increasing secretion of gonadotropins, a small number of follicles initially become recruited, followed by a larger number, and, finally, a small number respond to develop further. In response to intra- and extraovarian stimuli, recruited follicles might then be considered to be distributed normally. Although the trailing edge of recruited follicles is more difficult to delineate from the large pool of medium-sized follicles on Day 18, a small group (2–4) of follicles representing the leading edge of the distribution may have been identified (78). This small, but dominant, group of follicles secretes estradiol sooner than the other follicles and has been proposed to induce the ovulatory surge of luteinizing hormone (LH) to occur approximately 54 hr later (79).

The distribution of maturing, preovulatory follicles might become skewed because of two influences: atretic factors and a coordinated response to LH. Considerable atresia, particularly of the less mature follicles, occurs up to 4–5 hr before ovulation and results in a reduced number of follicles. Second, because hCG given to proestrus gilts can induce early ovulation, one might assume that some preovulatory follicles are able to respond to the ovulatory surge of LH before it is normally released. Therefore, although the small minority of more developed follicles on the leading edge of the normal distribution can induce the ovulatory surge of LH, perhaps those follicles, plus a large group toward the median of the distribution, also respond and ovulate more synchronously. Collectively, atretic forces on the trailing end and coordination of responses to LH on the leading edge of the once normal distribution could result in a skewed pattern of oocyte-follicular development.

The skewed pattern of follicular development results in a skewed pattern of ovulation and zygotic development in those females mated before ovulation. This skewed pattern of development continues to Day 12 when the more developed blastocysts of the litter synthesize and secrete estradiol into the uterine lumen before the less developed embryos. Exposing the progesterone-primed endometrium to estradiol at this time advances uterine secretion to the detriment of the less developed blastocysts of the litter. Events before ovulation can, therefore, be considered to effect which embryos will subsequently live or die; truly a situation in swine where embryogenesis recapitulates oogenesis.

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