

Control of ovulation rate in swine¹

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ABSTRACT: Follicular recruitment and atresia are important processes associated with ovulation rate in swine. Follicle-stimulating hormone regulates granulosa cell division, differentiation, and steroidogenic function, and, as such, significantly influences follicular growth and development. Follicle-stimulating hormone is an inducer of follicular recruitment in swine and an inhibitor of granulosa cell apoptosis, and it seems to be a major regulator of ovulation rate in swine. Although local factors, such as growth factors and steroid hormones, might regulate follicular development by controlling the expression of gonadotropin receptors or by modulating other related processes, the dominant role of FSH cannot be ignored. Recent results indicate that androgens might be among the local factors regulating

ovulation rate in swine. Administration of testosterone or the nonaromatizable androgen dihydrotestosterone increased the number of ovulations in gilts in a dose-dependent manner. Furthermore, administration of dihydrotestosterone increased the amounts of FSH receptor mRNA in pig preovulatory follicles. Steady-state amounts of FSH receptor mRNA are relatively high during the early follicular phase but decrease significantly as follicles grow and approach ovulation, suggesting that major changes in amounts of FSH receptor mRNA occur during late follicular development in pigs. Local ovarian factors that regulate follicular responsiveness to gonadotropins seem to be important components of the mechanisms that control ovulation rate in pigs.

Key Words: Ovarian Development, Ovulation Rate, Pigs

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J. Anim. Sci. 80(E. Suppl. 1):E36–E46

Introduction

A fundamental function of the ovary is to support development of oocytes. Oogenesis begins during fetal life and is highly influenced, later during the reproductive period, by follicular development. Beginning at puberty, about 10 to 25 follicles develop to the ovulatory stage and release oocytes every estrous period. The total number of ovulations is called the ovulation rate and it is an important parameter of swine reproductive efficiency. It should be noted that although ovulation rate is a limiting factor in determining the number of offspring born, numerous additional factors act upon the uterus and conceptuses throughout gestation and contribute to the number of healthy fetuses that develop to term.

Factors that alter ovulation rate might function by modulating, directly or indirectly, how many follicles

grow or how many follicles die (atresia). Follicular growth or atresia will be the main focus of the present review. Initially this review will describe follicular development and then will partition factors that affect recruitment from atresia. This overview will continue by reviewing some recent observations from our laboratory that suggest a novel role of androgens in regulating follicular development. Finally, a brief description of how some other factors might regulate recruitment or atresia will be discussed.

Folliculogenesis

Primordial follicles form the stock from which all follicles emerge (Peters, 1978). Approximately 500,000 primordial follicles are present in both ovaries by 10 d after birth in swine (Black and Erickson, 1968). Primordial ovarian follicles in this population are not stimulated to grow at the same time, and only a small number begin their development while the rest remain quiescent. In mammals, this characteristic increases the possibilities of having progeny throughout a rather long reproductive life.

Initiation of growth of primary follicles involves endocrine actions and regulatory effects of local factors from the somatic cells of the follicle (Hirshfield, 1991) and probably from the growing oocyte (reviewed by Picton

¹Salaries and research support provided by state and federal funds appropriated to The Ohio Agric. Res. and Dev. Center and The Ohio State Univ. Manuscript no. 16-01AS.

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Received April 5, 2001.

Accepted August 29, 2001.

et al., 1998). Early follicular growth is characterized by an increase in the number and layers of granulosa cells, which subsequently separate from each other resulting in formation of the antrum, a fluid-filled cavity (Zamboni, 1974). Initial studies using hypophysectomy techniques, and more recent experiments using knockout mice lacking a functional FSH receptor (**FSHR**) gene (Dierich et al., 1998), indicated that FSH seems to be absolutely necessary for antrum formation and post-antral follicular development. Therefore, development of responsiveness to FSH might be a key factor controlling follicular differentiation and function.

Growth rates of antral follicles of pigs and other species follow an exponential curve because of a dramatic increase in proliferation of granulosa cells and an increase in size of the antrum (Clark et al., 1975; Grant et al., 1989). Activated primary follicles of pigs require approximately 84 d for growing to the antral stage and an additional 19 d to grow to the preovulatory size of 10 mm (Morbeck et al., 1992). The rate of growth of porcine follicles from 3 to 10 mm in diameter has been estimated to be 1.14 mm/d (Dailey et al., 1976). During final maturation, cells of the theca and granulosa layers synthesize and secrete significant amounts of steroids, peptide hormones, prostaglandins, and other substances. These hormones are important because they participate locally in follicular development, and some of them convey signals that coordinate the functions of the hypothalamic-hypophyseal-ovarian axis.

Follicular recruitment refers to the formation of a pool of antral follicles from which the ovulatory follicle(s) is subsequently selected (Fortune et al., 1991). Follicular recruitment in pigs occurs between d 14 and 16 of the estrous cycle (Clark et al., 1982; Foxcroft and Hunter, 1985) or shortly after weaning. On d 16, approximately 40 to 50 follicles, 2 to 6 mm in diameter, are present in both ovaries (Grant et al., 1989). A number of these follicles are growing and represent the population of recruited follicles. Most follicles, and the oocytes they contain, degenerate and disappear from the ovaries through the process of atresia. Although atresia may occur any time during development of antral follicles, most follicles, for a given species, are lost during the transition from the small to large size (Fortune, 1994). Therefore, only a small proportion of recruited follicles (about 30 to 40%) are selected to complete final maturation and ovulate while the remaining become atretic, most of them before reaching 6 mm in diameter (Grant et al., 1989; Dailey et al., 1976). Ovulatory follicles are not readily identifiable until d 20 (Grant et al., 1989), which suggests that the process of selection is not completed until near ovulation. Therefore, the final number of ovulatory follicles is determined by how many follicles are recruited and by the ability of recruited follicles to continue to grow and avoid atresia. Because follicles might become atretic, even close to the time of ovulation, the process of selection probably takes place continuously, over the entire follicular phase (from luteolysis at approximately d 13 to 14 to ovulation; Foxcroft

et al., 1987). Follicular dominance, which occurs in cattle (Ko et al., 1991) and primates (Zelevnik and Kubik, 1986), has not been demonstrated in pigs.

Within each mammalian species, ovulation rate is regulated within a relatively narrow range. Changes in rates of follicular recruitment or atresia could alter the number of follicles that ovulate. This notion has been supported in recent studies. For instance, Meishan sows have a higher ovulation rate than Large White hybrids (27.7 vs 17.6 CL, respectively) and, probably related, Meishan sows have more follicles ≥ 2 mm on d 16 than Large White sows. Also, in Large White sows the number of follicles decreased from d 16 to 19, but this decrease does not occur in Meishan sows (Miller et al., 1998). Collectively, these observations suggest that the greater ovulation rates in the Meishan breed than in Large White hybrids is related to differences in both recruitment and atresia. Alternatively, the possibility that ovulation rate can be altered by manipulating the incidence of atresia alone has been shown in mice that exhibit increased ovulation rate and litter size following overexpression of the antiapoptotic factor bcl-2 (Hsu et al., 1996).

Follicle-Stimulating Hormone is an Effective Inducer of Follicular Recruitment

The number of follicles that are recruited might depend on concentrations of FSH or other regulatory factors, or on the number of follicles responsive to FSH present at the time of recruitment. Whether the latter is related to some endocrinological event occurring before recruitment, or what other factors might influence it, is not known. Several relationships support a regulatory role of FSH on follicular recruitment in swine, and some have been known for many years. Treatment with FSH or PMSG on d 15 and 16 of the estrous cycle increases the number of ovulations in gilts, but the effect significantly diminishes when given after d 16 (Hunter, 1979). Administration of FSH or PMSG increases ovulation rate in estrus-synchronized sows (Guthrie et al., 1997) and induces follicular recruitment in prepubertal gilts (Paterson, 1982) or during the luteal phase in post-pubertal gilts in a manner that is dose-dependent (Hunter, 1979). Partial suppression of FSH by administration of porcine follicular fluid to gilts during the early follicular phase reduced the subsequent number of small, medium, and large follicles, possibly due to inhibin present in follicular fluid (Guthrie et al., 1987). In another experiment, the numbers of medium-sized follicles were partially restored when FSH was given following treatment with porcine follicular fluid (Guthrie et al., 1988; Knox and Zimmerman, 1993). Active immunization against inhibin increased concentrations of FSH (area under the curve) by 27% and number of ovulations by 39% in gilts (King et al., 1993). A similar increase of 35% in ovulation rate was reported by other investigators in gilts immunized against inhibin (Brown et al., 1990). However, diminishing the

decrease in FSH that occurs after weaning in sows by passive immunization against inhibin did not alter numbers of piglets born (ovulation rate was not evaluated; Wheaton et al., 1998).

Despite the effects of exogenous FSH on ovulation rate, which indicate that it is a major inducer of follicular recruitment in pigs, a clear increase in FSH concentrations coincident with the period of follicular recruitment has not been detected in gilts (Flowers et al., 1989; Guthrie and Bolt, 1990) or sows (Shaw and Foxcroft, 1985; Foxcroft et al., 1987). A small increase in plasma FSH concentrations occurs around estrus in postpubertal gilts, coincident with the preovulatory LH surge, which is followed by a second increase approximately 72 h later. No significant changes in concentrations of FSH occur during the luteal phase until the time of luteolysis, when FSH concentrations decrease gradually through the period of estrus (Brinkley, 1981; Van de Wiel et al., 1981; Guthrie and Bolt, 1990; Guthrie et al., 1993). Likewise, during the follicular phase, FSH binding and FSH-stimulated adenylate cyclase activity by granulosa cells decrease as follicular size increases (Nakano et al., 1977; Lindsey and Channing, 1979). Moreover, amounts of FSHR mRNA determined using *in situ* hybridization (**ISH**) decrease from the time of recruitment to undetectable levels at estrus (Liu et al., 1998). In another study, FSHR mRNA became undetectable by ISH procedures when follicles reached 4 mm in diameter in sows after weaning (Liu et al., 2000). Experiments using reverse-transcription polymerase chain reaction (**RT-PCR**), a very sensitive technique, indicated that FSHR mRNA in surface walls of the largest follicles present from d 13, 15, and 17 of the estrous cycle were not different but decreased by d 19 (about fivefold relative to d 13, Cárdenas and Pope, unpublished data). This significant down-regulation of FSHR around estrus is only present in late-developing follicles, because small antral follicles permanently exhibit high amounts of FSHR throughout the estrous cycle (Yuan et al., 1996; Liu et al., 1998).

The decrease in plasma FSH concentrations and amounts of FSHR mRNA in late-developing follicles suggests a diminished influence of FSH on porcine follicles as they advance to the preovulatory stage. This does not necessarily mean that FSH is not needed for recruitment. The relatively greater concentrations of FSH before recruitment, and the concentrations present during recruitment, although decreasing, are probably enough to support follicular growth and function. For example, synthesis of estradiol, a process partially regulated by FSH, is still increasing even though FSH and FSHR mRNA are decreasing. The significance of the FSH decrease is not apparent. Perhaps, lesser concentrations of FSH would inhibit incorporation of more follicles into the cohort of recruited follicles, thus decreasing follicular heterogeneity. Reduced diversity among follicles is important to ensure uniform response to ovulation or decrease diversity in oocyte or embryonic development (Pope et al., 1990). Furthermore, de-

creased FSH stimulation might allow only those follicles that have reached certain developmental conditions to continue to grow, to avoid atresia, and to ovulate. Although this concept seems attractive as a mechanism of follicular selection in pigs, it will need to be examined further. As FSH stimulation becomes attenuated, additional support for follicular development could come from growth factors, LH, and steroid hormones.

Unlike FSH, LH remains almost unchanged during the follicular phase, except near the onset of estrus when the preovulatory LH surge occurs (Brinkley, 1981; Van de Wiel et al., 1981; Guthrie and Bolt, 1990). Luteinizing hormone binding in theca and granulosa cells increase as follicles become larger during the follicular phase (Nakano et al., 1977; Foxcroft and Hunter, 1985), increasing the ability of late-developing follicles to respond to LH. Amounts of LH receptor mRNA in granulosa and theca interna cells increases as follicles grow from 2 to 6 mm in diameter and then decrease in 8-mm preovulatory follicles (Liu et al., 2000), indicating that the LH receptor gene is down-regulated as follicles approach ovulation.

Specifically, what FSH does to stimulate follicular recruitment in swine is an important question. *In vitro* experiments conducted in swine or other species indicate that FSH might influence granulosa cell division by stimulating mitogen-activated protein kinases (Babu et al., 2000) or cell cycle regulators such as cyclin D2 (Sicinski et al., 1996). Also, FSH significantly stimulates estradiol synthesis by several mechanisms including regulation of expression of the CYP 19 gene that encodes for aromatase cytochrome P450 enzyme (Hickey et al., 1988), synthesis of progesterone (Ford and Howard, 1997), and expression of LH receptors, an important factor in the availability of aromatizable androgens. Inhibin secreted by maturing follicles is a potent enhancer of LH-stimulated androgen synthesis by theca cells (Hillier et al., 1994). Estradiol, in turn, stimulates granulosa cell proliferation, enhances the actions of FSH and LH on steroidogenesis (Daniel and Armstrong, 1980; Richards, 1980), and appears to be absolutely necessary for follicular growth and maturation (reviewed by Drummond and Findlay, 1999). Differences in litter size not necessarily related to ovulation rate have been associated to the chromosomal locus where the estrogen receptor alpha gene is located (reviewed by Rothschild et al., 1997).

Although FSH seems to be important in the control of follicular recruitment, a relationship between ovulation rate and peripheral concentrations of FSH has not been clearly demonstrated. For instance, lines of pigs differing in ovulation rate did not exhibit differences in LH, FSH, progesterone or estradiol during the estrous cycle (Mariscal et al., 1998). Similarly, no associations were found between plasma concentrations of FSH and ovulation rate in other species such as sheep (Gibbons et al., 1999). However, differences in the ability of follicles to respond to FSH through the influence of other

factors such as the number of FSHR might exist and could help to explain the differences in ovulation rate.

Cell signaling by FSH is mediated by a plasma membrane receptor, which is present exclusively in granulosa cells in females and is associated with the cAMP-protein kinase A and probably calcium second messenger systems (Flores et al., 1992; Simoni et al., 1997; Touyz et al., 2000). Factors that regulate expression of the FSHR gene have been investigated in different species. In cultured rat granulosa cells, amounts of FSHR mRNA were increased by treatment with FSH (Tilly et al., 1992b). The effect of FSH on FSHR mRNA was enhanced by IGF-I, which apparently occurred due to an increase in mRNA stability (Minigishi et al., 2000). In cultured granulosa cells of swine, small amounts of FSH increased FSHR mRNA (Sites et al., 1994); however, more recent results demonstrated that FSH, and other ligands that signal through cAMP, decreased steady state amounts of FSHR mRNA (Murphy and Dobias, 1999). Activin increases the number of FSH receptors in preantral follicles and prevents premature luteinization of large antral follicles (Findlay, 1993; Minigishi et al., 2000). Findlay (1993) speculated that the effect of activin on FSHR could be of particular importance for initiation of follicular development. In primates, testosterone administration increased FSHR mRNA in granulosa cells of primary follicles but did not alter FSHR mRNA in more advanced follicles (Weil et al., 1999). We have determined the effects of androgens on amounts of FSHR mRNA in porcine follicles and the results are discussed below.

Follicle-stimulating Hormone is an Inhibitor of Atresia in Granulosa Cells

Follicular atresia in swine (Tilly et al., 1991; Guthrie et al., 1995), and probably most mammals (Kaipia and Hsueh, 1997), is primarily induced by programmed cell death or apoptosis of granulosa and theca cells. Apoptosis is characterized by internucleosomal DNA fragmentation, cell shrinkage, plasma membrane blebbing, and formation of apoptotic bodies (Hsu and Hsueh, 2000). Although considerable progress has been made in understanding the mechanisms of cell death by apoptosis, the description of the apoptotic pathway in granulosa cells is not complete. Some results indicate that, as in other cell types, caspases are the final inducers of granulosa cell degradation (Hsu and Hsueh, 1998). The members of the bcl-2 family of apoptotic and antiapoptotic factors are upstream in this pathway and some of them have been shown to be functional in granulosa cells (Hsu and Hsueh, 1998).

The specific factors that induce atresia in porcine follicles have been difficult to identify. Initially, attempts to answer this question were made by determining hormonal changes in follicles undergoing atresia relative to healthy follicles. For example, atretic follicles had lower concentrations of estradiol and contained similar or greater concentrations of progesterone and

androgens in the follicular compared to nonatretic follicles (Maxson et al., 1985; Guthrie et al., 1993; Cárdenas and Pope, 1994). Atresia of pig follicles was also associated with a decrease in expression of mRNA for aromatase and gonadotropin receptors (Tilly et al., 1992a) and with a loss of aromatase activity (Maxson et al., 1985). Although these findings did not demonstrate cause-effect relationships, they seemed to indicate a role for estrogens and gonadotropins as inhibitors of atresia. This notion was supported by other experiments in which estrogen treatment decreased apoptosis in granulosa cells of rats while testosterone antagonized the effects of estrogens (Billing et al., 1993). More recently, Meishan sows that exhibited lesser rates of atresia than Large White hybrids during the follicular phase were observed to have greater concentrations of estradiol in follicular fluid (Miller et al., 1998).

Follicle-stimulating hormone decreased apoptotic DNA fragmentation by 60% and was regarded as a major suppressor of apoptosis in cultured rat granulosa cells or follicles (Chun et al., 1994, 1996). Likewise, FSH, as well as IGF-I, decreased DNA fragmentation in porcine granulosa cells in culture (Guthrie et al., 1998). Kaipia and Hsueh (1997) proposed that FSH could directly regulate apoptotic factors or influence other inhibitors of granulosa cell death or growth promoters. Recently, mcl-1, a new member of the bcl-2 family, inhibited apoptosis in rat granulosa cells, and amounts of its message were enhanced by gonadotropin treatment (Leo et al., 1999).

The Role of Androgens in Follicular Development and Ovulation Rate

Follicular theca and granulosa cells undergo steroidogenesis in a cooperative manner (Short, 1962). Aromatizable androgens (testosterone and androstenedione) are synthesized by thecal cells upon stimulation by LH, which then diffuse to granulosa cells for subsequent conversion into estradiol (pigs, Evans et al., 1981). In pigs, synthesis by theca cells and follicular fluid concentrations of androstenedione during the mid- and late-follicular phase are severalfold greater than those of testosterone (Evans et al., 1981; Tsang et al., 1985). The conversion of androgens into estradiol requires stimulation by FSH (Dorrington et al., 1975; Moon et al., 1975). Androstenedione can be converted into testosterone in a reversible reaction catalyzed by 17 β -hydroxysteroid dehydrogenase or aromatized to estrone, which then can be converted to estradiol-17 β . Testosterone is directly aromatized into estradiol-17 β (Peters and McNatty, 1980).

Evidence has accumulated to indicate that androgens play regulatory functions in follicular development. Androgens enhanced FSH-stimulated progesterone production and aromatase activity in rat and primate granulosa cells in vitro (Armstrong and Dorrington, 1976; Daniel and Armstrong, 1980; Harlow et al., 1986). Similarly, androgens stimulated [³H]thymidine incorpora-

Table 1. Effects of low amounts of androgens on numbers of corpora lutea and conceptuses or offspring in pigs

Treatment	Corpora lutea		Conceptuses or offspring		Reference
	Control	Androgen	Control	Androgen	
Testosterone (d 17 and 18, 1 mg/d)	14.4	16.7*	12.3 (d 11)	15.0*	Cárdenas and Pope, 1994
	—	—	9.6 (Total born)	10.7*	Unpublished
Testosterone (d 13 to estrus, 1 mg/d)	14.8	16.2*	12.8 (d 11)	15.3*	Cárdenas and Pope, 1997
	13.3	14.9*	12.5 (d 4–7)	13.6*	Cárdenas and Pope, 1997
Androstenedione (d 13 to estrus, 1 mg/d)	14.3	15.1	12.5 (d 11)	12.9	Cárdenas and Pope, 1997
DHT (d 13 to estrus, 6 µg/[kg BW·d])	17.4	20.3*	15.9 (d 11)	9.4*	Cárdenas, unpublished
DHT (d 13 to estrus, 60 µg/[kg BW·d])	19.5	26.6*	15.5 (d 3)	15.7	Cárdenas, unpublished

*Different from control ($P < 0.05$).

tion (Bley et al., 1997) and lipoprotein utilization (Schreiber et al., 1984) by granulosa cells. Androgens also stimulated development of mouse follicles in vitro (Murray et al., 1998), and administration of androgens to primates enhanced early development of ovarian follicles (Vendola et al., 1998, 1999). Moreover, we demonstrated for the first time that administration of androgens during the follicular phase increased ovulation rate in postpubertal gilts. A summary of results using small doses of androgens is presented in Table 1. In one experiment, testosterone administration on d 17 and 18 of the estrous cycle increased ovulation rate in gilts, apparently by decreasing the incidence of atresia (Cárdenas and Pope, 1994). In another experiment, a longer period of testosterone administration, from d 13 to estrus, not only increased ovulation rate, but also increased the percentage of blastocysts surviving to d 11 of gestation (Cárdenas and Pope, 1997). The total number of blastocysts was therefore increased by the combined effects of testosterone on ovulation rate and survivability of conceptuses. It is possible that factors that enhance follicular development, such as testosterone, could optimize oocyte growth, resulting in conceptuses with greater ability to survive.

The effect of testosterone on ovulation rate was also observed when follicular recruitment and ovulation were artificially induced during the luteal phase, indicating that this effect of testosterone is not necessarily related to or influenced by the physiological conditions present during the follicular phase. In this experiment, gilts were administered daily injections of vehicle or 1 mg of testosterone on d 11 to 16 or 4 to 9 of the estrous cycle. Follicular recruitment was induced the 1st d of testosterone treatment using PMSG, and follicles were induced to ovulate by a single injection of hCG, 72 h later. Testosterone treatment increased ($P < 0.05$) the number of induced corpora lutea when treatment began on d 4, but the effect ($P = 0.09$) was not clear when testosterone treatment began on d 11 (Table 2). Superimposing testosterone treatment on ovulation induction using PG600 in prepubertal gilts did not alter ovulation rate but had a significant effect on increasing embryonic survival (Table 3). An important practical question has been whether the increase in ovulation rate and embryonic survival produced by treatment with testosterone

would be translated into more piglets born. Results (unpublished) of a field trial indicated that treatment of multiestrous gilts with vehicle (85 gilts treated and mated) or 1 mg of testosterone (84 gilts treated and mated) on d 17 and 18 of the estrous cycle increased litter size by approximately one pig (9.6 in vehicle- vs 10.7 in testosterone-treated gilts, $P = 0.03$). Farrowing rates (gilts farrowing/gilts treated and mated) were 80 and 76.2% in vehicle- and testosterone-treated gilts, respectively.

It is possible that the effects of testosterone on ovulation rate described above could have been mediated by estradiol. Alternatively, it might be possible that testosterone, independent of estradiol, acting via the androgen receptor (AR), enhanced transcription of certain genes involved in follicular development. In addition, androgens have been shown to induce rapid changes in intracellular Ca^{+2} in luteinizing granulosa cells (Machelon et al., 1998). Effects of this nature are so-called nongenomic and apparently do not involve the AR; however, participation of membrane receptors has been proposed for nongenomic actions produced by other steroids (reviewed by Wehling, 1997). Nongenomic actions of androgens have not been demonstrated in vivo, and it is not clear whether they could be involved in the effects of testosterone observed in our experiments.

Kreider et al. (2001) recently reported that active immunization against androstenedione increased ovulation

Table 2. Numbers of induced corpora lutea (CL) in gilts treated with vehicle or testosterone coincident with induction of ovulation using PMSG (750 IU) and hCG (750 IU) during the luteal phase of the estrous cycle

Beginning of treatment	Vehicle	Testosterone
Day 11 ^a	15.7 ± 2.4	22.4 ± 2.6
Day 4 ^b	5.9 ± 1.1 ^c	11.9 ± 2.5 ^d

^aExperiment 1. Gilts ($n = 10$ per treatment) received PMSG on d 11, hCG on d 14, and daily doses of 1 mg of testosterone on d 11 to 16. Induced CL were counted on d 23.

^bExperiment 2. Gilts ($n = 18$ per treatment) received PMSG on d 4, hCG on d 7, and daily doses of 1 mg of testosterone on d 4 to 9. Induced CL were counted on d 12.

^{c,d}Means within a row with different superscripts differ ($P < 0.05$).

Table 3. Corpora lutea (CL) and d-11 blastocysts in prepubertal gilts induced to ovulate with PG600 and treated daily with 1 mg of testosterone from day of PG600 administration to mating

Treatment	Number of CL	Number of blastocysts	Blastocyst survival, %
Vehicle (n = 19)	16.1 ± 1.3	11.4 ± 1.3	71.3 ± 6.1 ^a
Testosterone (n = 19)	16.7 ± 1.6	14.5 ± 1.6	85.6 ± 3.2 ^b

^{a,b}Means within a column with different superscripts differ ($P < 0.05$).

rate in gilts by approximately three corpora lutea (ovulations). It is not clear how immunization against androstenedione, a weak androgen that can be converted into testosterone and estrogens in ovarian follicles, would increase ovulation rate in pigs. In a previous experiment, active immunization against androstenedione increased ovulation rate in gilts, did not affect concentrations of FSH and LH, but increased concentrations of androstenedione in follicular fluid (McKinnie et al., 1988). Perhaps it can be speculated that an increase in androstenedione would increase testosterone and estradiol in the follicular fluid, which in turn would stimulate follicular development.

The AR and receptors for other steroid hormones, thyroid hormones, retinoids, and vitamin D are structurally related proteins that regulate gene transcription (Tsai and O'Malley, 1994). A long (A, 110 kDa) and a short (B, 87 kDa) form of AR, having similar functional activities, are present in different organs and are derived from differences in translation initiation of the AR mRNA (Gao and McPhaul, 1998). Similar to that in rats and primates (Tetsuka et al., 1995; Hillier et al., 1997), the AR protein in swine ovaries was localized mainly in granulosa cells of small and medium-sized follicles during the 1st wk following ovulation (Garret and Guthrie, 1996). We determined the relative amounts of the AR protein and mRNA in ovarian follicles of swine from d 13 to 19 of the estrous cycle using immunohistochemistry and RT-PCR. These preliminary results (Cárdenas and Pope, unpublished data) suggested that AR protein and mRNA are 1) present in maturing follicles during the follicular phase and 2) their relative amounts do not seem to change during follicular growth from recruitment to the preovulatory stage.

To examine the involvement of the AR in ovulation rate in pigs, we administered daily injections of 0, 6, 60, or 600 µg/kg BW of dihydrotestosterone (DHT) from d 13 to estrus. Dihydrotestosterone binds and activates the AR, but unlike testosterone it cannot be aromatized due to 5 α -reduction of the A ring (Wilson, 1975). Ovulation rate significantly increased by approximately 3, 10, and 17 with each additional dose of DHT. However, the number of d-11 blastocysts drastically decreased as dose of DHT increased and no blastocysts were recovered in gilts that received the highest dose of DHT (Cárdenas et al., 2002). Low recovery rates of blastocysts were associated with opaque uterine flushings, indicating that uterine function could be altered by overexposure to DHT. In a subsequent experiment, it was determined

whether the effects of DHT on ovulation rate were associated with specific periods of the follicular phase. Gilts received daily i.m. injections of vehicle from d 13 to estrus or they received 60 µg/kg of DHT from d 13 to estrus (entire follicular phase), d 13 to 16 (follicular recruitment), or d 17 to estrus (postrecruitment). Results demonstrated that all three periods of DHT administration increased ovulation rate and that numbers of embryos in gilts treated from d 13 to estrus were not different compared with controls but increased in gilts treated from d 13 to 16 or d 17 to estrus (Cárdenas et al., 2002).

In another trial, gilts received daily injections of vehicle or 6 µg/kg BW of testosterone or DHT from d 13 to estrus. Tissue from the largest follicles was collected the 1st d of estrus and processed for determination of AR and FSHR mRNA by RT-PCR. Amounts of AR mRNA were not altered by androgen treatment. However, a significant increase (about onefold) in amounts of FSHR mRNA occurred in gilts treated with DHT compared with those that received vehicle (Cárdenas et al., 2002).

These results using DHT demonstrate that stimulation of AR, probably at the ovary, is capable of altering late follicular development, resulting in increased ovulation rate. The effects of DHT were more pronounced relative to those of testosterone, which might be explained by the greater androgenic activity of DHT compared with testosterone in other cell types. This effect of DHT is unique, and based on present knowledge only treatment with FSH, eCG or, in the recent study, DHT has increased ovulation rate at this magnitude in pigs. Although the mechanisms involved in this effect of DHT are probably numerous, the up-regulation of FSHR mRNA might be one of the mechanisms in place. An effect of this kind would increase the ability of follicles to respond to the stimulatory effects of FSH during recruitment or to continue growing and be selected into the ovulatory pool, when otherwise they could become atretic. These effects could also increase the estrogenic activity of growing follicles due to the effects of FSH on aromatase. The relationships just described might be helpful in understanding the control of ovulation rate in pigs and are summarized and integrated with previous knowledge in Figure 1.

Other Factors That Alter Ovulation Rate May Modulate the Actions of Major Regulators of Follicular Growth or Atresia

It is well recognized that nutritional conditions may affect reproductive performance of domestic mammals

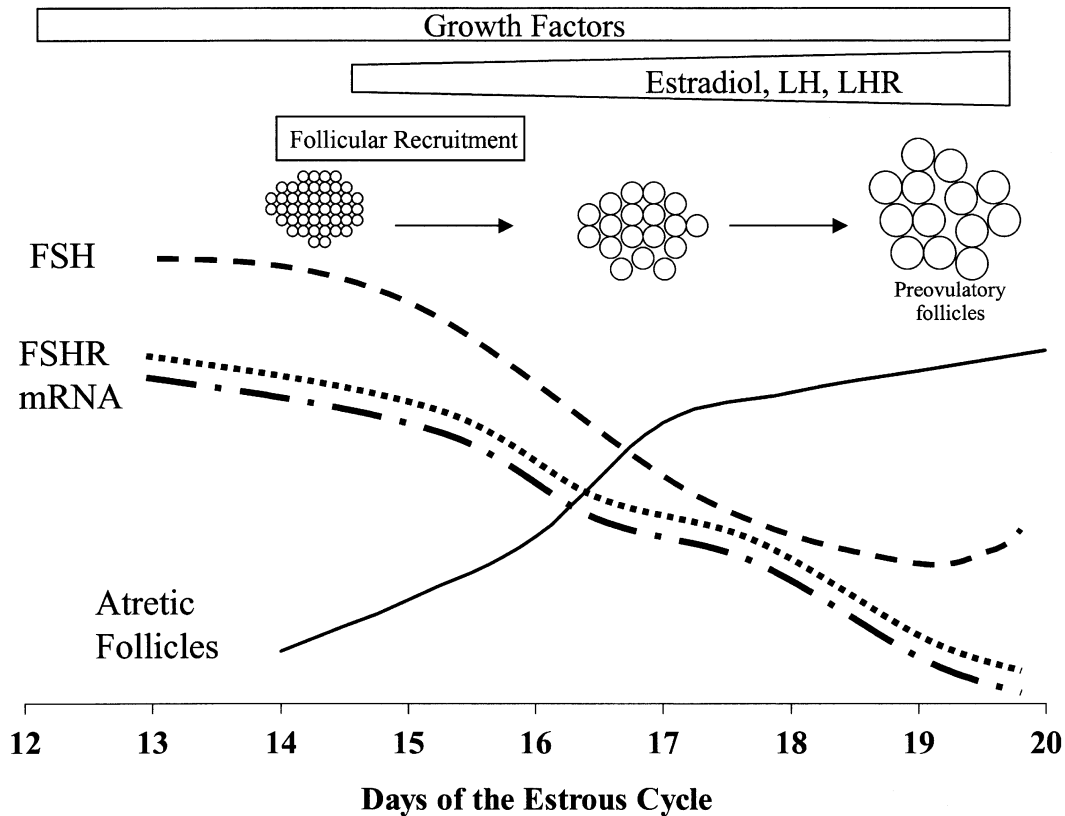


Figure 1. Follicle-stimulating hormone (FSH) is a major factor that stimulates follicular growth and induces follicular recruitment during the early follicular phase (d 14 to 16 of the estrous cycle) in swine. Recruited follicles continue their phase of rapid growth and most of them (60 to 70%) become atretic before d 17. The number of recruited follicles and incidence of atresia are key determinants of the number of follicles that ovulate. Follicular growth following recruitment might continue to be supported by FSH; however, other factors such as luteinizing hormone (LH), estradiol, and growth factors possibly become increasingly important due to decreasing concentrations of FSH and amounts of FSH receptor (FSHR). Up-regulation of FSHR (dotted line) might be a way to increase responsiveness to FSH, resulting in enhanced follicular growth and decreased atresia.

including swine, and that maintaining normal levels of nutrition is important for optimal ovarian function. Under certain conditions, particularly related to animal age, follicular development becomes responsive to changes in feed intake. For instance, increasing feed intake (flushing) in pubertal gilts increased ovulation rate, but the treatment was less effective in postpubertal gilts (Rhodes et al., 1991). In the review by Prunier and Quesnel (2000), it was concluded that in postpubertal gilts, nutrition restriction consistently decreased ovulation rate when applied during the luteal and follicular phases; however, in most experiments with sows, nutrition restriction did not alter ovulation rate when imposed during lactation or after weaning. Recent experiments partially support these conclusions. For instance, in gilts that were fed a high plane of nutrition during the entire estrous cycle or were restricted during d 1 to 7 or 8 to 15, ovulation rate was not altered but embryonic survival by d 28 was lower in gilts restricted from d 8 to 15 than in the other groups (Almeida et al., 2000). In another experiment, 50% restriction or 125% overfeeding during lactation in primiparous sows did

not alter ovulation rate or embryonic survival (Zak et al., 1997, 1998). Likewise, administration of GnRH every 6 h during feed restriction from d 22 to 28 of lactation failed to affect ovulation rate, embryonic survival, or plasma concentrations of progesterone in primiparous sows (Mao et al., 1999).

The effects of nutrition on ovulation rate might be mediated by changes in secretion of growth factors, metabolic hormones, and gonadotropins (Flowers et al., 1989; reviewed by Cox, 1997) and/or their interactions. Components of the IGF system have been identified in ovarian follicles of swine and have been shown to be associated with follicular growth (Mondschein et al., 1991; Yuan et al., 1996, Liu et al., 2000) and partial inhibition of follicular atresia (Guthrie et al., 1998). Transforming growth factor β has also been shown to influence granulosa cells (Gangrade and May, 1990; Chang et al., 1993). Interestingly, although there have been some indications that administration of insulin increases ovulation rate, the results have not been consistent (reviewed by Cox, 1997). Administration of insulin to primiparous sows after weaning tended to in-

crease the number of follicles on d 5 but reduced concentrations of estradiol, IGF-I, and IGF-I mRNA in large follicles (Whitley et al., 1998b). In a recent study (van den Brand et al., 2000a), changes in plasma insulin concentrations were induced by feeding sows a high- or low-energy diet (with fat or starch as energy source) during a 21-d lactation period. Postprandial plasma glucose and insulin were greater and ovulation rate tended to be greater in sows fed the starch diet than in sows fed the fat diet, and there was no association between glucose or insulin and LH concentrations. In a follow-up experiment (van den Brand et al., 2000b) ovulation rate tended to be greater in sows fed the high-energy than in those fed the low-energy diet. These results indicate that increasing dietary energy, and the consequent increase in insulin, did not clearly affect ovulation rate. Similarly, ovulation rate was not altered when insulin was administered for 4 d to primiparous sows beginning the day after weaning (Whitley et al., 1998a) or when feed-restricted primiparous sows received insulin for 5 d during the weaning period (Quesnel and Prunier, 1998). Although altering nutrition has not been able to consistently change ovulation rate in sows, some follicular characteristics were altered, and it is not clear whether these changes could perhaps affect oocyte development.

Differences in ovulation rate between breeds, or lines within breeds, have been demonstrated. Ovulation rate can respond to selection and lines of pigs with greater ovulation rate than others have been developed (Johnson et al., 1999). Selection for FSH concentrations to increase ovulation rate was estimated to be effective and more practical than direct selection on ovulation rate (Cassady et al., 2000). This supports the importance of FSH actions on follicular development and ovulation rate.

Implications

Increasing ovulation rate has the potential to increase swine reproductive efficiency. Identification of important regulators of ovulation rate will help us to design systems for hormonal supplementation, nutritional manipulation, or genetic selection directed to improve the quantity and quality of oocytes released during the ovulatory process. Although our knowledge of ovulation rate in swine is far from complete, experimental results indicate that FSH and ovarian factors that regulate its actions (hormone receptors, growth factors and their receptors, steroid hormones, and enzyme activities) seem to be critical in determining the number of follicles that ovulate. Investigative efforts need to continue to focus on understanding FSH secretion and its action on target cells of the ovarian follicle to better control follicular development and ovulation rate.

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