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Minireview

Proteolytic Degradation of Insulin-Like Growth Factor Binding Proteins by Ovarian Follicles: A Control Mechanism for Selection of Dominant Follicles[1]

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ABSTRACT

This review summarizes evidence for the role of proteolytic enzymes that degrade and inactivate insulin-like growth factor binding proteins (IGFBP) during follicular development in mammals. In some species (e.g., bovine), evidence indicates that decreases in IGFBP-4 and -5 levels in estrogen-dominant preovulatory follicles are likely due, in part, to increased protease activity, whereas lower levels of IGFBP-2 are not due to increased proteolysis. Increased IGFBP-4 and -5 protease along with lower amounts of IGFBP-4 binding activity and greater amounts of free IGF-I are some of the earliest developmental changes documented in bovine growing antral follicles. This protease activity has recently been ascribed to serine metalloprotease(s), including pregnancy-associated plasma protein-A (PAPP-A), which was first detected in human follicular fluid nearly 20 yr ago. Other recent studies verified the presence of PAPP-A mRNA in granulosa cells of humans, monkeys, cattle, mice, and pigs. Increases in the amount of PAPP-A mRNA in granulosa cells during follicular development occurs in some but not all species, indicating that other proteases or protease inhibitors may be involved in IGFBP degradation. Whether the hormonal control of PAPP-A production/activity by the ovary differs between monotocous and polytocous animals will require further study. These protease-induced decreases in IGFBP-4 and -5 likely cause increased levels of bioavailable (or free) IGFs that stimulate steroidogenesis and mitogenesis in developing dominant follicles, which ultimately prepare the follicle(s) and oocyte(s) for successful ovulation and fertilization.

follicle, follicular development, granulosa cells, growth factors, ovary

INTRODUCTION

Insulin-like growth factor-I (IGF-I) and IGF-II increase mitogenesis and synergistically augment the stimulatory effects of gonadotropins on steroidogenesis of ovarian cells in vitro [for review, see 1–4]. Whether IGF-I or -II is the primary trophic stimulus to the ovary may depend on the species because granulosa cells of human follicles contain IGF-II mRNA and not IGF-I mRNA [2, 4–6], whereas granulosa cells of rats contain IGF-I and not IGF-II mRNA [2, 7, 8] and bovine granulosa cells contain both [2, 9]. Published data indicate that both IGF type I and II receptors are present in granulosa and theca cells but IGF-II is a less effective stimulator of ovarian cell steroidogenesis than IGF-I due to its weaker competition for the type I IGF receptor [2, 10, 11]. Nonetheless, with an ED_{50} of IGF-II on ovarian cell steroidogenesis ranging between 1.4 and 37 ng/ml [10–13], increased free IGF-II as well as IGF-I may further enhance follicular cell differentiation. In most of the mammalian species evaluated, follicular growth and atresia are associated with changes in follicular fluid (FF) insulin-like growth factor binding protein (IGFBP) levels more than with changes in total FF concentrations of IGF-I or -II [1–4, 14, 15]. There are at least six different IGFBPs (22–45 kDa), all of which are high-affinity carrier proteins that bind IGF-I and IGF-II, prolong their half-lives, and in most situations, block their action [for review, see 2, 16–18]. Specifically, in cultured granulosa and thecal cells, IGFBPs have the ability to inhibit the synergistic effects of IGF-I and gonadotropins on steroidogenesis [19–23]. In some cell types, IGFBPs may have direct IGF-independent effects [17, 18], but whether these are operative within ovarian cells is unknown. Levels (as measured by ligand blotting and thus measuring binding activity) of IGFBP-2, -4, and -5 in FF decrease during follicular growth and development of dominant follicles, and increase during atresia in cattle [2, 15, 24], sheep [3, 25], pigs [26–28], humans [4, 29], and horses [14, 30]. In contrast, levels (as measured by ligand blotting and thus measuring binding activity) of IGFBP-3, the predominant IGFBP in FF, during follicular growth remain constant [30] or increase [14] in horses and are unchanged in cattle [15, 24, 31, 32] and humans [29]. Affinities of IGFBP-2 and -5 for IGF-II are 3- to 10-fold greater than for IGF-I, whereas affinities of IGFBP-3 and -4 for IGF-II are similar to those for IGF-I [2, 16, 17, 33]. Thus, ligand blotting techniques using only ^{125}I-IGF-I may not be as sensitive as using ^{125}I-IGF-II to detect some of the IGFBPs (e.g., IGFBP-5). Because mRNA for most of the IGFBPs (i.e., IGFBP-1 through -6) have been identified in ovarian tissue of a variety of mammalian species [for review, see 1–4, 34], it is likely that some of the changes in FF IGFBP levels are due to changes in local synthesis of the IGFBPs. The main cell layers of antral follicles (i.e., granulosa and theca) contain IGFBP mRNA and produce IGFBPs in vitro, but which cell layer produces a particular IGFBP is species specific. For example, equine granulosa cells produce only IGFBP-2 and -5 [14]; murine [8, 35, 36]

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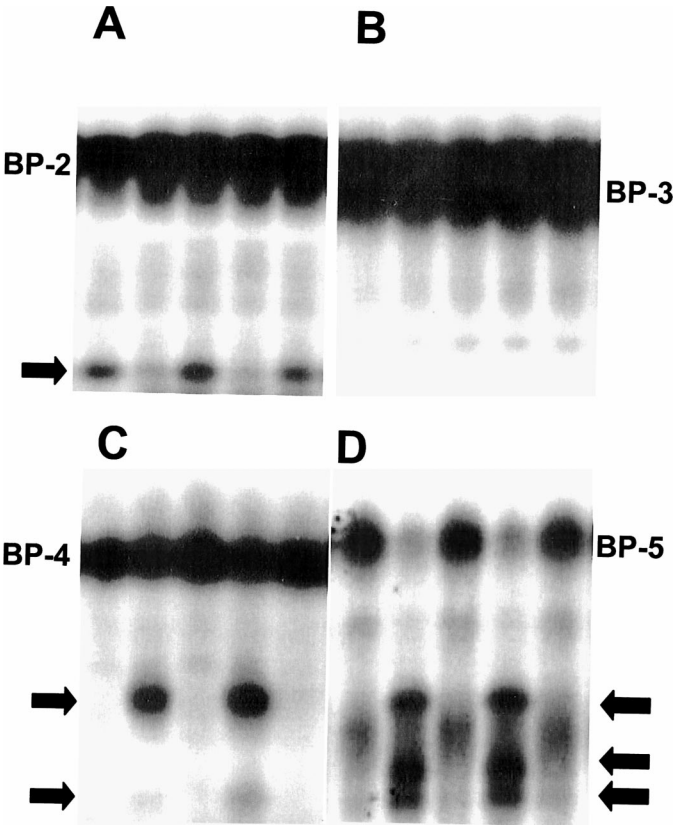

FIG. 1. Representative autoradiograph measuring proteolytic activity of bovine follicular fluid (FF) to recombinant human ^{125}I-labeled IGFBP-2 (**A**), -3 (**B**), -4 (**C**), and -5 (**D**). Lanes 1–2: FF samples from small subordinate and large dominant follicles of cow 684; lanes 3–5: FF samples from small subordinate, large dominant, and small subordinate follicles of cow 275. Arrows indicates breakdown products: **A**, 12 kDA; **B**, none; **C**, 10 and 15 kDa; **D**, 10, 12, and 15 kDa. (Modified from Spicer et al. [48], with permission from Domest Anim Endocrinol.)

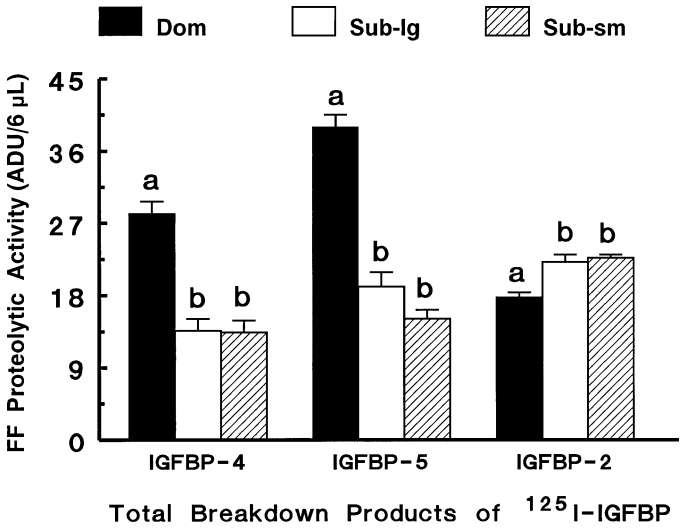

FIG. 2. Summary of densitometric scans of bands of breakdown products for ^{125}I-IGFBP-4, -5, and -2 (n = 6 animals) and expressed as arbitrary densitometric units (ADU) per 6 µl of follicular fluid. ^{a,b} Within each IGFBP, means without a common superscript differ ($P < 0.05$). Follicles were classified as small (sm), large (lg), subordinate (Sub), and large dominant (Dom) from preovulatory dairy cows. ^{125}I-labeled IGFBPs were incubated with FF and ^{125}I-labeled breakdown products (see arrows in Fig. 1) were quantified densitometrically; ADU of multiple bands (i.e., breakdown products) were arithmetically added for a combined proteolytic activity. (Modified from Spicer et al. [48], with permission from Domest Anim Endocrinol.)

and bovine [37, 38] granulosa cells produce primarily IGFBP-2, -4, and -5; and porcine granulosa cells produce IGFBP-2, -3, -4, and -5 [39–41]. Physiological changes in ovarian IGFBP mRNAs have been evaluated semiquantitatively via in situ hybridization in several species and data indicate that granulosa-cell IGFBP-4 mRNA increases during atresia in rats [34, 35], and decreases during development of large antral follicles in humans [42] and rats [8, 34]. In addition, the hormonal regulation of follicular production of IGFBPs appears species and ovarian-cell specific. For example, FSH regulates IGFBP-4 and -5 production by murine [8, 36, 43] and porcine [39] but not bovine [37, 38, 44] granulosa cells. Recently reported, LH and estradiol decrease IGFBP-4 production by bovine granulosa cells whereas LH and estradiol have no effect on IGFBP-4 production by thecal cells [38]. Therefore, evidence exists to support the notion that the changes in IGFBPs found in FF are due, in part, to changes in local (i.e., granulosa or theca) synthesis. However, IGFBPs transudating from the blood cannot be ruled out as another source of FF IGFBP and is more difficult to test experimentally. A third cause for changing levels of IGFBP in FF is posttranscriptional modification via proteolytic degradation of the IGFBP [for review, see 17, 45]. Several studies [14, 46–53] have reported changes in intrafollicular IGFBP-2, -3, -4, and -5 proteolytic activity during development of dominant preovulatory follicles (Figs. 1 and 2) in several species (see next section), indicating that changes in intraovarian levels of specific IGFBPs may be regulated by changes in local synthesis as well as proteolysis. In bovine thecal cells, LH increases IGFBP-4 mRNA levels [44] but has no effect on IGFBP-4 production [38], a result likely due to increased proteolysis of IGFBP-4. To complicate matters, inhibitors to these IGFBP proteases may exist and be hormonally and temporally regulated (see next section). As with the IGFBPs, specificity of ovarian cell (i.e., granulosa and theca) IGFBP protease activity/production may be species specific. This review will focus on recent evidence documenting changes in intraovarian IGFBP-2, -3, -4, and -5 proteolysis during development of dominant follicles in cattle and other species and discuss possible enzymes involved and their regulation.

IGFBP PROTEASES IN OVARIAN FOLLICULAR FLUID AND THEIR CHANGES DURING FOLLICULAR DEVELOPMENT

Intraovarian IGFBP-4 and -5 Proteolysis

As summarized in Table 1, IGFBP-4 and -5 proteolysis by FF has been documented in several species including cows (Figs. 1 and 2 [48–51]), mares [14], ewes [3, 46, 52], sows [3, 47], and women [4, 53]. Proteolysis of IGFBP-2 by FF has also been reported in cattle (Figs. 1 and 2; [48, 54]), sheep [46], and pigs [47]. No proteolysis of IGFBP-3 occurred in the presence of bovine (Fig. 1; [48]), equine [14], porcine [47], or human [4, 53]) FF, whereas FF IGFBP-3 proteolytic activity decreased during follicular growth in ewes [46] (Table 1). A 1994 study [55] first reported that a non-IGF-I binding fragment (approximately 21 kDa) for IGFBP-5 is present in bovine FF using immunoblotting techniques, but whether this fragment was generated from within the follicle or transudated from serum was not determined. In cattle, as in most species evaluated, FF concentrations of IGFBP-5 are over 2-fold greater

TABLE 1. Summary of the relative changes in IGFBP-2, -4, and -5 protease activity in FF and granulosa cell PAPP-A mRNA in ovarian follicles during follicular growth and atresia in various species.*

Species	Follicle type	PAPP-A protein	PAPP-A mRNA	Proteolysis of IGFBP-4	Proteolysis of IGFBP-5	Proteolysis of IGFBP-2
Woman	Growing	Increased [108–110]	Unchanged [99]	Increased [53]	?	?
	Atretic	Decreased [108–110]	Decreased [99]	Decreased [53]	?	?
Monkey	Growing	?	Unchanged [111]	?	?	?
	Atretic	?	?	?	?	?
Cow	Growing	?	Increased [101] Unchanged [112]	Increased [48, 78, 101]	Increased [48, 78]	Decreased [48]
	Atretic	?	Decreased/unchanged [101, 112]	Decreased [48, 101]	Decreased [48]	Unchanged [48]
Mare	Growing	?	?	Increased [14]	Increased [14]	Unchanged [14]
	Atretic	?	?	Decreased [14]	Decreased [14]	Unchanged [14]
Sow	Growing	?	Increased ? [101]	Unchanged [47]	Increased [47]	Increased [47]
	Atretic	?	Decreased ? [101]	Unchanged [47]	Decreased [47]	Decreased [47]
Mouse	Growing	?	Increased [98]	?	?	?
	Atretic	?	Undetectable [98]	?	?	?

* ? = Not determined/uncertain; numbers in brackets are reference numbers.

than those of IGFBP-4, and both of their concentrations are much less than those of IGFBP-2 and -3 [15, 24, 48, 55]. Thus, changes in concentrations of FF IGFBP-4 and/or -5 would likely result in a small change in the concentration of bioavailable or free IGF-I and -II, requiring the follicle to be exquisitely sensitive to IGF-I or II (see next section). Proteolysis of ^{125}I-IGFBP-4 and -5 are negatively correlated with FF levels of IGFBP-4 and -5 ($r = -0.6$ to -0.8) and positively correlated with FF estradiol ($r = 0.7$–0.9) and androstenedione ($r = 0.5$–0.7) levels [48]. These observations imply either or both steroids may regulate IGFBP-4 and -5 levels via inhibition of their production, induction of IGFBP protease activity, and/or inhibition of production of IGFBP protease inhibitors such as tissue inhibitor of metalloprotease-1 (TIMP-1 [56, 57]). In support of steroid control of IGFBP production, Spicer and Chamberlain [38] observed that estradiol directly inhibited IGFBP-4 production by bovine granulosa cells in vitro, but it was not determined if this decrease in production was due to a change in proteolysis of IGFBP-4. The hormones IGF-I, FSH, and/or estradiol [20, 58] induce IGFBP-4 proteolysis in cultured human granulosa cells. Also, FSH induces an IGFBP-4 and -5 protease in rat granulosa cells in vitro [59–62], and the IGFBP-5 protease was inhibited by IGF-I or IGF-II [61]. Similarly, IGF-I diminished IGFBP-5 proteolysis by human osteocarcinoma cells [63] and ovine chondrocytes [64] and diminished proteolysis of IGFBP-3 by cultured porcine granulosa cells [65]. In contrast, IGF-I activates IGFBP-4 protease in cultured uterine myometrial cells [66] and enhances IGFBP-4 proteolysis by ovarian FF [49], and IGF-II enhances IGFBP-4 proteolysis by human pregnancy serum [67]. In view of data of Mazerbourg et al. [49] indicating that IGF-I enhances IGFBP-4 degradation by bovine, porcine, and equine FF, it is likely that IGF-I and -II directly enhance IGFBP-4 protease activity present in ovarian follicles rather than indirectly via IGF-I induction of steroidogenesis [68, 69]. Additional data of Qin et al. [70] indicate that IGF-II binding to IGFBP-4 makes IGFBP-4 more susceptible to proteolysis rather than IGF-II directly interacting with the IGFBP-4 protease. Other hormones, including cytokines and glucocorticoids, increased IGFBP-5 proteolytic activity in conditioned medium from smooth-muscle cells [71], but whether the inhibitory effect of cortisol on bovine granulosa cell IGFBP-5 accumulation in vitro [37] is due to increased IGFBP-5 proteolysis or decreased IGFBP-5 gene expression remains to be determined. Recent data documenting the hormonal regulation of ovarian IGFBP-specific protease mRNA will be summarized in the next section.

Intraovarian IGFBP-2 Proteolysis

A 1994 study [55] reported that two non-IGF-I binding fragments (22 and 14 kDa) for IGFBP-2 are present in bovine FF using a combination of immuno- and ligand-blotting techniques, but whether these fragments were generated from within the follicle or transudated from serum was not determined. More recently, evidence for a small but significant amount of IGFBP-2 proteolytic activity in bovine preovulatory follicles was found when measuring appearance of a 12-kDa breakdown product of ^{125}I-IGFBP-2 (Fig. 1 [48]). As summarized in Figure 2, large dominant bovine follicles had significantly lower IGFBP-2 proteolytic activity, as measured by appearance of this 12-kDa fragment, than subordinate small or large follicles [48]. Also, proteolysis of ^{125}I-IGFBP-2 was negatively correlated with FF estradiol ($r = -0.68$) and positively correlated with FF levels of IGFBP-2 ($r = 0.55$) (unpublished observations). These results are the opposite of that observed for IGFBP-4 and -5 proteolytic activity [48]. In contrast, there is a small increase in IGFBP-2 proteolytic activity during follicle growth in sheep [46] and pigs [47] detected by immunoblotting, but no change during follicle growth in horses [14], indicating that species differences in FF IGFBP-2

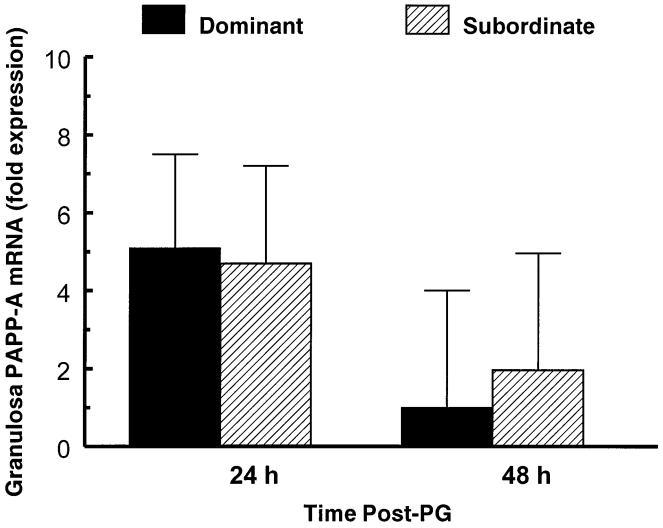

FIG. 3. PAPP-A mRNA levels measured by quantitative real-time RT-PCR in granulosa cells collected from preovulatory dominant and subordinate bovine follicles 24 and 48 h after prostaglandin-$F_{2\alpha}$ (PG)-induced luteolysis. Values are means ± SEM of 4–6 follicles and expressed as fold of lowest mean. No significant differences were noted.

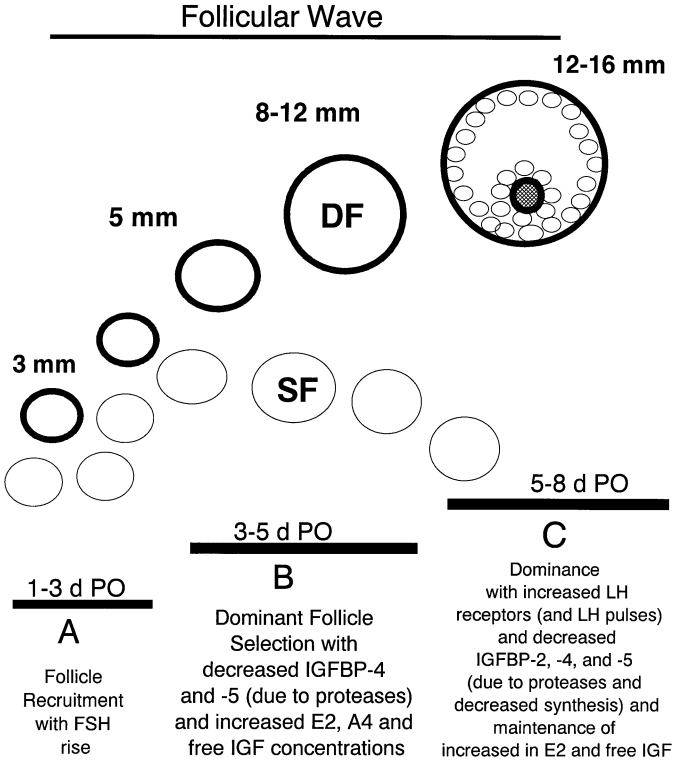

FIG. 4. Hypothesized role of IGFBPs and their proteases during follicular development in cattle. Phase A: (Day 1–3 postovulation [PO]) small follicle PAPP-A gene expression (and hence IGFPB-4 proteolysis) in granulosa cells is stimulated by FSH while thecal IGFBP-4 gene expression is stimulated by LH. Phase B: (Day 3–5 PO) the selected follicle's PAPP-A activity is further enhanced by increased free IGF-I and -II, which further increases estradiol (E2) and androstenedione (A4) production, which increases tissue kallikreins activity (and hence increased IGFBP-5 proteolysis). Phase C: (Day 5–8 PO) dominant follicle IGFBP-2, -4, and -5 mRNA and tissue kallikrein mRNA in granulosa cells is suppressed (and hence decreased IGFBP-2 proteolysis) by LH and estradiol. DF, dominant follicle; SF, subordinate follicle.

proteolysis may exist (Table 1). In these previous studies [48, 54], IGFBP-2 proteolytic activity was much weaker than that of IGFBP-4 and -5. Furthermore, proteolytic cleavage of IGFBP-2 by pregnancy-associated plasma protein-A (PAPP-A) took longer to develop than that of IGFBP-4 [48, 54], suggesting that PAPP-A may be required to activate another enzyme that then degrades IGFBP-2 (see next section). Differences in techniques may also account for some of the discrepancies in the literature. Degradation of ^{125}I-labeled IGFBPs is considered one of the more precise methods of detecting IGFBP proteolysis [45], and antibodies used for immunoblotting may not recognize proteolytic fragments or may cross-react with other IGFBPs [17, 24, 72]. Further research will be required to elucidate the differences in FF IGFBP-2 proteolysis among species.

IDENTIFICATION AND HORMONAL REGULATION OF OVARIAN IGFBP PROTEASES

Ovarian IGFBP-4 and -5 Proteases

The IGFBP protease(s) detected in porcine FF was inhibited by metalloprotease inhibitors (e.g., EDTA) but not serine protease inhibitors [47]. However, a serine protease, similar to plasmin, produced by porcine granulosa cells, degraded IGFBP-3; degradation of other IGFBPs was not evaluated [65]. Why IGFBP-3 proteolysis by porcine FF was not detected [47] while porcine granulosa cells produce IGFBP-3 protease activity [65] is unknown, but may indicate other factors, such as an endogenous IGFBP-3 protease inhibitor [56, 57], TIMP-1, are present in FF [73–76] but may not be produced in vitro. Using various pharmacologic enzyme inhibitors, the protease that degrades IGFBP-3, -4, and -5 found in sheep FF has been identified as a serine metalloprotease [46, 52]. The IGFBP protease in bovine [48, 77, 78] and equine [14, 49] FF also has characteristics of a serine metalloprotease and has been hypothesized to be pregnancy-associated plasma protein-A (PAPP-A) a pappalysin and member of the metzincin protease family [49, 53, 77–79]. In order for PAPP-A to cleave IGFBP-4, IGF-I or -II must be present [49, 67, 70]. In mares, the serine metalloprotease degrades IGFBP-5 but not IGFBP-2 and -3, and is inhibited by two kallikrein-specific inhibitors [14]. A protease produced by rat granulosa cells that degrades IGFBP-5 but not IGFBP-1, -2, -3, -4, or -6 was not inhibited by serine protease inhibitors or two metalloprotease (MMP) inhibitors, TIMP-1 and -2 [61, 80], indicating that species differences may exist in terms of which specific protease exists in the follicle and which specific IGFBP(s) is (are) targeted by these proteases in FF. It should be emphasized that many of these studies used single doses of the various inhibitors and evaluated single substrates (e.g., IGFBP-4), which may have incompletely characterized the enzyme activity. Indeed, the specificity of the IGFBP protease is likely due to differential expression of one or more of the numerous types of metalloproteases (e.g., MMP-1, -2, -9, -13 [for review, see 81–83]) and/or serine proteases (e.g., plasmin, PAPP-A, kallikrein [for review, see 84, 85]) among species. In addition to PAPP-A, PAPP-A2 (a pappalysin and member of the metzincin protease family), cleaves IGFBP-5 and is not dependent on IGF-I/-II for its activity as is PAPP-A [86, 87]. As mentioned, another serine metalloprotease family, the kallikreins [84, 85, 88–90], are present in FF [91, 92] and may degrade several of the IGFBPs, including IGFBP-2 [93, 94], IGFBP-3 [93, 95, 96], and IGFBP-5 [14, 48]. At least 15 specific kallikrein genes have been identified in humans [85, 88–90]. It is clear from work in nonovarian tissues that there is frequent

coexpression of many kallikreins in the same tissues, and this may point to a functional relationship among various proteolytic enzymes [88]. In particular, kallikreins may activate one or more MMPs [97], which then may cleave IGFBPs. Moreover, mRNA for PAPP-A [98–101], MMPs [82, 83], and various kallikreins (e.g., K2–4, K6–9, K12 [102–107]) have been detected in ovarian tissues. In mice, exogenous PMSG treatment increased whole ovarian PAPP-A mRNA levels by 5-fold within 24 h; levels returned to baseline by 48 h, attesting to the fugacious character of PAPP-A mRNA regulation [98]. In situ hybridization studies with rodents and humans revealed the granulosa as the main cell layer of PAPP-A mRNA localization within the follicle, although the corpus luteum also exhibited strong PAPP-A gene expression [98, 99]. Interestingly, the presence of PAPP-A in human FF was first discovered nearly 20 yr ago [108–110]. In these early studies, it was reported that FF concentrations of PAPP-A and estradiol were significantly correlated [108, 109]. Similarly, a more recent study using quantitative reverse transcription-polymerase chain reaction (RT-PCR) showed a significant positive correlation ($r = 0.73–0.79$) between granulosa cell aromatase mRNA and PAPP-A mRNA levels measured in variously sized healthy and atretic bovine and porcine follicles [101]. However, little or no differences in levels of PAPP-A mRNA in granulosa cells exist between small and large healthy bovine and porcine follicles [101]. Consistent with the latter observation, Zhou et al. [111], using in situ hybridization of monkey ovaries, recently discovered that PAPP-A mRNA was widely expressed in granulosa cells of follicles of all sizes with no apparent correlation between PAPP-A and LH receptor mRNA intensity. Also, hCG treatment of monkeys increased IGFBP-4 mRNA levels in theca cells but had no effect on PAPP-A mRNA levels in granulosa cells [111]. We find no difference in PAPP-A mRNA levels in granulosa cells between preovulatory dominant and subordinate follicles in cattle (Fig. 3 [112]) and no correlation between levels of PAPP-A mRNA and IGFBP-4 mRNA or protein in granulosa cells (unpublished data). Interestingly, a recent immunolocalization study using human ovaries indicated that granulosa, theca, and luteal cells stained for PAPP-A [113]. Therefore, either PAPP-A gene expression is so acutely regulated that differences among follicle classes are missed or other proteases and/or their inhibitors are involved in IGFBP degradation. Further work will be required to elucidate these possibilities, as well as determine whether the hormonal control of PAPP-A production/gene expression by the ovary differs between monotocous and polytocous animals. If PAPP-A is involved in selection of dominant follicles, then differences among monotocous and polytocous animals would be expected.

Ovarian IGFBP-2 Proteases

As summarized in the previous section and Table 1, proteolysis of IGFBP-2 by FF from dominant follicles has been reported for cattle [48, 113], sheep [46], and pigs [47], but not mares [14]. A recent report indicates that the IGFBP-2 proteolytic activity in bovine FF, detected by immunoblotting, may be due to PAPP-A [54]. Also, IGFBP-2 proteolysis decreased in dominant versus subordinate bovine follicles [48]. If PAPP-A is responsible for IGFBP-2 degradation and PAPP-A activity increases as dominant follicles develop [77, 78, 101], then other factors must be involved in regulating IGFBP-2 levels in FF [48]. Evidence for several possibilities exist: 1) intraovarian (i.e., granulosa or theca) production of IGFBP-2 may decrease [37, 38], 2) increased heparin-binding fragments from degraded IGFBP-5 may block PAPP-A cleavage of IGFBP-2 [54, 96], 3) decreased intrafollicular IGF-II [2, 15] may reduce the effectiveness of PAPP-A on IGFBP-2 [94, 114], and/or 4) decreased production of other intraovarian IGFBP-2-specific proteases, such as kallikreins [106], may occur. Because levels of estradiol and androstenedione were negatively correlated with FF IGFBP-2 levels in bovine [48] and equine [14], further research should focus on the role that these hormones play in regulating follicular IGFBP-2 production and gene expression. In addition, studying the presence and regulation of other enzymes known to cleave IGFBPs such as the disintegrin metalloproteases (e.g., ADAM [115–117]) and the complement serine proteases (e.g., C1 [71, 118, 119]) within the ovary will likely be fructuous areas of future research. Regarding the latter class of proteases, a recent study indicated that a portion of the dexamethasone-induced increase in IGFBP-5 protease activity in vitro was due to increased release of C1-inhibitor by human fibroblasts [71]. Elucidation of ovarian IGFBP-specific protease inhibitors should also be a focus of future research.

CONCLUSIONS AND FUTURE DIRECTIONS

In conclusion, levels of IGFBP-4 and -5 proteases increase in large preovulatory follicles coincident with a reduction in IGFBP-2, -4, and -5 levels and an increase in FF estradiol and androstenedione (Fig. 4). In contrast, little or no IGFBP-2 and -3 protease activity exists in FF of preovulatory or subordinate follicles of most species. Thus, low amounts of IGFBP-4 and -5 in dominant follicles may be a result of enzyme degradation, whereas loss of IGFBP-2 in preovulatory dominant follicles is not. Furthermore, greater FF proteolysis of IGFBP-4 and -5 along with lower binding activity (i.e., levels) of IGFBP-4 and greater amounts of free IGF-I (i.e., 2–9 ng/ml [78, 120, 121]) are the earliest developmental changes that have been documented in bovine follicles >6 mm in diameter [32, 78, 120]. Aromatase activity in bovine follicles is exquisitely responsive to low concentrations of IGF-I in the presence of FSH (i.e., ED_{50} = 5–6 ng/ml [68]), and this effect supports the hypothesis that small increases in free IGF-I are sufficient to stimulate the process of follicular differentiation (Fig. 4). Future research should focus on identifying the species-specific hormonal regulators of IGFBP-4- and -5-specific protease(s) in FF as well as further characterize the temporal interactions that may exist among the various proteases and their inhibitors present within the ovarian follicle during its development.

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