Xenopus laevis Ovarian CYP17 Is a Highly Potent Enzyme Expressed Exclusively in Oocytes

EVIDENCE THAT OOCYTES PLAY A CRITICAL ROLE IN XENOPUS OVARIAN ANDROGEN PRODUCTION

We show here that XeCYP17 has high 17α-hydroxylase activity that rivals their 17α-hydroxylase activity. Furthermore, expression levels of other important steroidogenic enzymes in various ovarian cell types have not been examined.

We show here that XeCYP17 has high 17α-hydroxylase, and even more pronounced 17,20-lyase, activities in both the Δ5 (pregnenolone) and Δ4 (progesterone) pathways (Fig. 1). This differs from most known CYP17 isoforms, which generally favor one pathway over the other, and rarely have 17,20-lyase activity that rivals their 17α-hydroxylase activity. Furthermore, we show that ovarian CYP17 activity is present exclu-

The phenomenon of steroid-induced maturation of Xenopus oocytes has served as a model for studying cell cycle and non-genomic steroid signaling for several decades (1–5). During this time, progesterone has been considered the primary physiologic mediator of oocyte maturation, perhaps through interactions with classical progesterone receptors expressed in the oocyte (6, 7). We have recently shown, however, that testoster-

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Pathway: 

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\begin{align*}
\text{Cholesterol} & \xrightarrow{\Delta5} \text{Pregnenolone} \\
\text{Pregnenolone} & \xrightarrow{\text{CYP17}} \text{Androstenedione} \\
\text{Androstenedione} & \xrightarrow{\Delta4} \text{Testosterone} \\
\text{Androstenedione} & \xrightarrow{\Delta4} \text{Androsterone} \\
\text{Pregnenolone} & \xrightarrow{\Delta4} \text{Progesterone} \\
\text{Progesterone} & \xrightarrow{17\alpha,20\beta\text{-lyase}} \text{17\alpha-hydroxyprogesterone} \\
\text{17\alpha-hydroxyprogesterone} & \xrightarrow{\text{CYP17}} \text{17\alpha}-\text{hydroxypregnenolone} \\
\text{17\alpha}-\text{hydroxypregnenolone} & \xrightarrow{\text{CYP17}} \text{Pregnenolone} \\
\text{Pregnenolone} & \xrightarrow{\text{CYP17}} \text{17\alpha-hydroxypregnenolone} \\
\text{17\alpha-hydroxypregnenolone} & \xrightarrow{\text{CYP17}} \text{17\alpha-hydroxyprogesterone} \\
\text{17\alpha-hydroxyprogesterone} & \xrightarrow{\text{CYP17}} \text{17\alpha-hydroxypregnenolone} \\
\text{17\alpha-hydroxypregnenolone} & \xrightarrow{\text{CYP17}} \text{Pregnenolone} \\
\end{align*}
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Fig. 1. Ovarian steroid biosynthesis. The sex steroid synthesis pathway, including several key enzymes, is shown. CYP17 is highlighted with a box, and the 17α-hydroxylase and 17α,20β-lyase activities are indicated on the right. The Δ5 and Δ4 pathways are indicated on the top, with the Δ5 steroids on the left and Δ4 steroids on the right.

Separatively in oocytes, whereas other important steroidogenic enzymes, including 3β-HSD and 17β-HSD, are located primarily in the surrounding follicular cells. Finally, we propose a "two-cell" model for androgen synthesis in the Xenopus ovary that involves both oocytes and follicular cells. This model implies that germ cells themselves are critical for Xenopus ovarian androgen production, which in turn may play an important physiologic role in promoting their own maturation.

EXPERIMENTAL PROCEDURES

Oocyte and Follicular Cell Preparation—Ovaries were harvested from Xenopus laevis (Nasco, Fort Atkinson, WI) and oocytes were isolated by incubation with 1 mg/ml collagenase A (Roche Molecular Biochemicals) at room temperature for 4 h in modified Barth's solution (MBSH) as previously described (14, 15). Oocytes were then washed and incubated overnight at 16 °C in MBSH with 1 mg/ml bovine serum albumin, 1 mg/ml Ficoll, 100 units/ml penicillin, and 0.1 mg/ml streptomycin. Stage V–VI oocytes were selected and examined microscopically to confirm the absence of follicular cells. Follicular cells were isolated by incubating ovaries in collagenase as above for 1 h. Oocytes were allowed to settle by gravity for ~5 min, and the supernatant containing follicular cells was then centrifuged at 800 x g for 5 min. The pelleted cells were washed 3 times with MBSH, and any remaining oocytes were removed manually under a dissecting microscope. Similar numbers of follicular cells were used in all of the metabolism experiments. In addition, nearly identical results were obtained using follicular cells removed from oocytes after up to 4 h of treatment with collagenase, or using follicular cells separated from oocytes by incubation with trypsin (16).

Preparation of Oocyte Membranes—Crude oocyte membranes were prepared as previously described (17). In short, stage V–VI oocytes were homogenized in membrane buffer (83 mM NaCl, 1 mM MgCl2, 1 mM phenylmethylsulfonyl fluoride, 0.5 μg/ml aprotinin, 0.5 μg/ml leupeptin, 0.5 μg/ml pepstatin, 10 mM Hepes, pH 7.6) at 4 °C. The homogenate was centrifuged at 800 x g for 5 min and the supernatant containing the membranes was removed and centrifuged two more times at 800 x g. The supernatant was then centrifuged at 15,000 x g for 15 min, and the membrane pellet was resuspended in membrane buffer. Membranes were centrifuged twice more at 15,000 x g and resuspended in MBSH. Protein concentrations were then measured using the BCA kit (Pierce), and samples were frozen at ~80 °C until needed. We saw no significant drop in CYP17 activity after a single freeze/thaw cycle of the membranes.

CYP17 Enzyme Assays in Oocyte Membranes—17α-hydroxylase and 17α,20β-lyase activities in oocyte membranes were performed as previously described (18). Membranes were assayed under initial rate kinetics by incubation in 50 mM potassium phosphate buffer (pH 7.4) with 0.03–1 μM steroid (Sigma and Steraloids, Newport, RI) and 1 mM of the cofactor NADPH in a 500-μl total volume at 16 °C for 30 min. Each reaction contained 50 μg of membranes and 50,000 cpm of [7-14C]pregnenolone (19), [1,2,6,7-3H]pregnenolone, [1,2,6,7-3H]androsterone, or [1,2,6,7-3H]17α-hydroxyprogesterone (PerkinElmer Life Sciences). Steroids were extracted with 3 ml of 3:2 ethyl acetate/hexane. Amounts of radioactivity were measured using a scintillation counter, with >90% recovery from medium. Steroids were concentrated under nitrogen and separated by TLC using 3:1 chloroform/methanol acetate. Quantitation of radioactivity was measured by cutting out the steroid spots on the TLC plate and measuring radioactivity using liquid scintillation (20). Kinetic behavior was approximated as a Michaelis-Menten system for data analysis. The identities of all steroids in these and the other metabolism experiments were confirmed by high performance liquid chromatography.

Sex Steroid Precursors Pregnenolone and Progesterone Are Rapidly Metabolized to Testosterone by Xenopus Ovaries—Previous work in our laboratory demonstrated that β-HCG or pregnant mare serum gonadotropin (PMSG) stimulation of Xenopus ovaries in vivo and in vitro produced high levels of testosterone and small to moderate amounts of androstenedione. In contrast, little to no androgen precursors, including pregnenolone, 17OHProg, progesterone, and 17OHProg, were detected (8). To determine whether Xenopus ovaries could convert sex steroid precursors to androgens in the absence of gonadotropins, we examined pregnenolone and progesterone metabolism by incubating ovarian tissue with the respective radiolabeled steroids. Both pregnenolone and progesterone were rapidly converted to testosterone (Fig. 2), with more than 90% loss of each steroid by 2 h. Virtually no intermediates or further metabolites of testosterone (e.g. estrogen or dihydrotestosterone) were detected in both the pregnenolone- and progesterone-treated ovaries (<10% of total counts at all time points). These data suggest that Xenopus ovaries contain all of the enzymatic machinery necessary for the conversion of sex steroid precursors to testosterone independent of gonadotropin stimulation. Furthermore, this ability to rapidly metabolize pregnenolone and progesterone likely explains why both steroids are nearly undetectable in gonadotropin-stimulated ovaries both in vivo and in vitro (8).

CYP17 in Xenopus Oocyte Membranes Possesses High 17α-Hydroxylase and 17α,20β-Lyase Activity in Both the Δ5 and Δ4 Pathways—The rapid conversion of progesterone and pregnenolone to androgens implies that the Xenopus ovary contains CYP17, the first key enzyme in the conversion of these sex steroid precursors to androgens (22, 23). Indeed, we previ-
profiles of the *Xenopus* and human CYP17 enzymes, and to confirm that the observed enzymatic activities in the *Xenopus* oocyte membranes were in fact mediated by the cloned XeCYP17 enzyme, we expressed the human and *Xenopus* CYP17 proteins individually in HEK-293 cells. Fig. 4, A and B, shows that XeCYP17 converted progesterone to 17OHPreg, and then to AD (40% of total counts by 8 h), confirming that both 17α-hydroxylase and 17,20-lyase activities are carried by this protein. In contrast, HuCYP17 contained high 17α-hydroxylase, but very little 17,20-lyase, activity in the Δ4 pathway (Fig. 4, A and C, 6% conversion to AD by 8 h). Calculated *Km* values for HuCYP17-mediated 17α-hydroxylase reactions in the Δ4 and Δ5 pathways were ~0.54 and 0.28 μM, respectively (data not shown), which correlate well with published values (18). This confirms that the enzyme is functioning as expected in our HEK-293 expression system.

Comparison of the calculated lyase/hydroxylase *V* \(_{\text{max}}\) ratios in HEK-293 cells confirmed that XeCYP17 had high 17,20-lyase activity in both pathways, with lyase/hydroxylase *V* \(_{\text{max}}\) ratios of approximately unity (Fig. 4D). In contrast, the 17α-hydroxylase reaction was dominant in both pathways for the HuCYP17, with ratios of ~0.3 in the Δ5 pathway and ~0.05 in the Δ4 pathway. The lower lyase/hydroxylase ratios of XeCYP17 expressed in the HEK-293 cells when compared with the oocyte membranes could be because of many factors, including the availability of important cofactors such as NADPH and the flavoprotein reductase(s), or the species compatibility of the 17,20-lyase cofactor cytochrome b\(_{5}\) in human *versus Xenopus* tissues. The 17,20-lyase activity in HEK-293 cells was still relatively high, however, thus these experiments appear to confirm that the cloned XeCYP17 enzyme is indeed responsible for the potent 17,20-lyase activity seen in oocyte membranes. Notably, similar results were qualitatively seen in COS cells; however, the presence of endogenous CYP17 in these cells precluded their use for quantitative studies.

*Xenopus* Oocytes Possess High CYP17 Activity, but Little to No 3β-HSD or 17β-HSD Activity—Having established that *Xenopus* CYP17 can metabolize sex steroid precursors equally well in both the Δ4 and Δ5 pathways, we next determined which cells within the ovary contained CYP17 activity. As mentioned, we had previously shown that isolated *Xenopus* oocytes possess high CYP17 activity in the Δ4 pathway (8). To confirm the presence of CYP17 in *Xenopus* oocytes, we separated oocytes from surrounding follicular cells and performed steroid metabolism experiments. Notably, isolated oocytes were examined very carefully both under the dissecting microscope and by staining of oocyte sections to exclude the presence of follicular cell contamination in our preparations. Fig. 5 represents one of over 50 different hematoxylin/eosin-stained oocyte sections, with no detectable follicular cell contamination.

As expected, isolated oocytes contained high CYP17 activity in both the Δ5 and Δ4 pathways, as radiolabeled pregnenolone and progesterone were rapidly metabolized to only DHEA and AD, respectively (Fig. 6A). Interestingly, the isolated oocytes did not appear to significantly express any of the other enzymes responsible for the various stages in the production of sex steroids. For example, 3β-HSD is responsible for the conversion of Δ5 to Δ4 steroids (Fig. 1). The rapid conversion of radiolabeled pregnenolone to DHEA in the absence of detectable Δ4 steroid production (Fig. 6A, left panel) argues strongly that oocytes lack significant 3β-HSD activity. Likewise, 17β-HSD is necessary for the conversion of DHEA to androstenediol, and of androstenedione to testosterone (Fig. 1). The lack of detectable androstenediol in the pregnenolone-treated cells, as well as the near absence of testosterone in progesterone-treated cells (<10% of the total counts at all time points measured), sug-
gests that oocytes contain little 17β-HSD activity (Fig. 6A). Finally, stimulation of isolated oocytes with β-HCG did not promote significant steroid production by radioimmunoassay (data not shown), suggesting that they contain little CYP11A1 or steroidogenic acute regulatory protein (stAR) activity, both of which are necessary to augment formation of pregnenolone (Fig. 1).

Xenopus Ovarian Follicular Cells Contain 3β-HSD and 17β-HSD Activities, but No CYP17 Activity—If oocytes have no significant 3β-HSD and 17β-HSD activity, then these enzymes must be present in the surrounding ovarian follicular cells to complete ovarian androgen synthesis. In support of this hypothesis, small amounts of radiolabeled pregnenolone were converted to progesterone by isolated ovarian follicular cells, indicating the presence of 3β-HSD in these cells (Fig. 6B, left panel). Similar numbers of follicular cells converted radiolabeled DHEA to AD at a significantly higher rate (Fig. 6B, middle panel), thus confirming the presence of 3β-HSD and suggesting that DHEA may be preferred over progesterone as a substrate for Xenopus ovarian 3β-HSD. Finally, radiolabeled AD was very efficiently converted to testosterone (Fig. 6B, middle and right panels), thereby demonstrating the presence of 17β-HSD in the follicular cells as well. Surprisingly, follicular cells did not hydroxylate progesterone or pregnenolone at all (Fig. 6B, left panel, and data not shown), indicating that ovarian CYP17 activity is contained exclusively in the oocytes themselves.

Because separation of oocytes and follicular cells revealed differential expression of the steroidogenic enzymes, the isolated cell types were recombined to determine whether the complete steroidogenic pathway from pregnenolone to testosterone could be reconstituted. The recombination of follicular cells and oocytes resulted in testosterone production (Fig. 6C), confirming that these two populations of cells were indeed

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**Table 1**

| Pathway          | Δ5  | Δ4  | Activity          |
|------------------|-----|-----|-------------------|
| A: PREG          |     |     | 17α-hydroxylase   |
| B: PROG          |     |     | 17α, 20-lyase     |
| C: 17OHPREG      |     |     |                   |
| D: 17OHPROG      |     |     |                   |

**Figure 3**

Representative Lineweaver-Burk plots of 17α-hydroxylase and 17,20-lyase activities in oocyte membranes. Oocyte membranes were prepared and treated as described under “Experimental Procedures.” Lines were derived from least-squared fit to the data points, with \( r^2 \) values are indicated. Starting steroids are indicated, with the Δ5 steroids on the left (A and C) and Δ4 steroids on the right (B and D). 17α-Hydroxylase reactions are represented on the top (A and B), whereas 17,20-lyase reactions are shown on the bottom (C and D).
sufficient to mediate complete steroidogenesis. Several intermediate steroids were also produced, including progesterone, DHEA, and AD, suggesting that the reconstituted system was less efficient than the intact ovary in producing testosterone.

**Blockade of Androgen Production Attenuates β-HCG-stimulated Maturation of Xenopus Oocytes in Intact Ovarian Follicles**—To confirm the physiologic importance of CYP17-mediated androgen production in Xenopus oocyte maturation, intact ovarian follicles were stimulated with β-HCG alone or in combination with the potent CYP17 inhibitor VN/85-1. The maxi-
TABLE II
Inhibition of β-HCG-induced oocyte maturation in Xenopus ovaries

Ovarian fragments were treated with 100 units/ml of β-HCG in the presence of the indicated antagonist and steroids. Steroids were extracted from the medium and mature oocytes were counted after 12 h. Values are expressed as mean ± S.D. (n = 3). Similar experiments were performed 3 times with ovaries from three different frogs with nearly identical results.

| Antagonist                  | Inhibition of maturation | Steroid concentration |
|-----------------------------|---------------------------|------------------------|
|                             | %                         | Testosterone | AD | Progesterone |
| Ethanol                     | 0                         | 95 ± 10      | 57 ± 2.2 | 1.3 ± 0.26 |
| 500 nm VN/85–1             | 28 ± 5.3                  | 2.3 ± 2.4  | 6.0 ± 4.6 | 7.1 ± 3.0 |
| 500 nm VN/85–1 + 500 nm AD | 106 ± 10                  | 81 ± 8.1 | 9.7 ± 3.6 |
| 500 nm VN/85–1 + 500 nm progesterone | 4.0 ± 1.0 | 13 ± 5.5 | 147 ± 6 |

![CYP17 phylogenetic tree](image)

**Fig. 7.** CYP17 phylogenetic tree. The neighbor joining method was used to create a CYP17 phylogenetic tree based on amino acid sequence homology (MacVector, Accelrys, San Diego, CA). The x axis represents relative evolutionary distance. See text for details.

**DISCUSSION**

Ovarian sex steroid production is essential for follicular growth and subsequent ovulation in nearly every animal (25–27). In frogs and fish, these steroids also appear to be critical regulators of oocyte maturation (28, 29), which is defined as the resumption of meiosis from prophase I to metaphase II. Fish oocyte maturation is regulated by various hydroxylated progesterone metabolites (30, 31), whereas testosterone may be an important physiologic mediator of *Xenopus* oocyte maturation (8). Sex steroids may be involved in higher vertebrate oocyte maturation as well; however, evidence for or against such a role is still minimal at this point in time.

Sex steroid production requires the cytochrome P450 enzyme CYP17 to catalyze both its 17α-hydroxylase and 17,20-lyase reactions, respectively. Whereas all mammalian CYP17 isoforms have relatively equal 17α-hydroxylase activities in both the Δ4 (progesterone) and Δ5 (pregnenolone) pathways, most have preferential 17,20-lyase activity for one of the two pathways. For example, in small mammals, such as rats (32), mice (33), hamsters (34), and guinea pigs (35), CYP17 is more active in the Δ4 pathway. In contrast, human and primate CYP17s prefer the Δ5 pathway (18, 20). We have found that XeCYP17 has high 17,20-lyase activities in both the Δ4 (progesterone) and Δ5 (pregnenolone) pathways (Table I), suggesting that, unlike most mammals, *Xenopus* frog ovaries can utilize both pathways to produce sex steroids. Furthermore, the rate of the XeCYP17-mediated 17,20-lyase reaction equals (HEK-293 cells, Fig. 4D) or exceeds (oocyte membranes, Fig. 3 and Table I) that of the 17α-hydroxylase reaction in both pathways. These high lyase activities dramatically contrast with nearly every mammalian isoform of CYP17, where the 17α-hydroxylase activities markedly exceed the 17,20-lyase activities (18, 36) (Fig. 5D).

Interestingly, the eel, fish, and shark CYP17 enzymes, which are most closely related to XeCYP17 by phylogenetic analysis (Fig. 7), appear to have robust 17,20-lyase activities in both pathways as well (37, 38), although no exact measurements of 17,20-lyase activity have been reported.

Taken together, these data lead us to speculate that the level of CYP17-mediated 17,20-lyase activity, as well the preferential use of one or both (Δ4 and Δ5) of the steroidogenic path-

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*W. H. Yang, L. B. Lutz, and S. R. Hammes, unpublished data.*
ways, may reflect the need of the enzyme to produce reproductive steroids versus cortisol in various organisms. In humans, for example, cortisol is essential for normal growth and development. Cortisol is derived primarily from the 21-hydroxylation of 11α-hydroxyprogesterone; thus, the relatively low 17,20-lyase activities of human CYP17, which leads to the accumulation of 17α-hydroxylated steroids, may be necessary to permit cortisol production in human adrenal glands. In contrast, frogs, as well as sharks, eels, and most fish, do not appear to need cortisol; instead, they rely on corticosterone or corticosterone metabolites as their primary glucocorticoids (39–41). CYP17 is therefore not required for glucocorticoid production in these lower vertebrates; its sole function appears to be to generate sex steroids. In short, CYP17 may have evolved from favoring primarily sex steroid production in lower vertebrates, which do not require cortisol, to promoting both cortisol and sex steroid production in higher vertebrates. Accordingly, the lower vertebrate CYP17s appear to form a closely related phylogenetic family by sequence homology, whereas the more evolved higher vertebrate CYP17s appear to be part of a distinctly separate family of proteins (Fig. 7).

The mechanism behind the unusually high 17,20-lyase activity of XeCYP17 relative to other CYP17 isoforms is an intriguing issue. One clearly important factor that regulates 17,20-lyase activity of human CYP17 is the presence of the co-factor protein cytochrome b$_5$ (18, 42). Perhaps the high 17,20-lyase activity of XeCYP17 is because of differences in the influence of or dependence on cytochrome b$_5$ as a cofactor for this reaction. Alternatively, Xenopus CYP17 may contain sequences that enhance its ability to bind to 17-hydroxylated steroids or other important cofactors. Given its relatively high 17,20-lyase activity, the Xenopus isoform may serve as a useful tool in teasing apart the mechanisms controlling the two enzymatic activities of the CYP17 enzyme.

The exclusive expression of XeCYP17 in oocytes suggests an unusual mechanism of sex steroid production driving oocyte maturation in the frog ovary. Taking into account the results reported in this study, we propose a model for steroid biosynthesis and steroid-induced maturation of oocytes (Fig. 8). In this model, pregnenolone would be produced in the follicular cells. Because pregnenolone is inefficiently converted to progesterone (Fig. 6), even in the presence of a CYP17 inhibitor (Table II), very little progesterone is likely being produced by 3β-HSD at any time. Because the follicular cells do not express CYP17, pregnenolone must then enter the surrounding oocytes to be converted to DHEA. Additionally, because CYP17 is equally active in the Δ4 pathway, any small amounts of progesterone that are produced by the follicular cells would be rapidly converted to AD in the oocyte, thus further preventing significant accumulation of progesterone. DHEA and AD would then be transported back to the follicular cells, where 17β-HSD and 3β-HSD would complete testosterone synthesis. Finally, testosterone from the follicular cells would re-enter the oocyte to promote its maturation. Because AD is also capable of promoting maturation, one cannot rule out the possibility that AD also plays a role in oocyte maturation in vivo; however, given the significantly higher potency and ovarian concentrations of testosterone relative to AD (8), testosterone is most likely the primary physiologic mediator of maturation in Xenopus oocytes. Although progesterone appears capable of promoting maturation in vitro, the lack of significant progesterone production at all times by β-HCG-stimulated frog ovaries, even in the presence of a CYP17 inhibitor, argues against a major role for progesterone in oocyte maturation in vivo.

Our model bears some similarity to the two-cell models put forth to explain sex steroid biosynthesis in other systems (43, 44). In this case, we do not actually know how many different cell types exist within our follicular fraction; however, it is quite clear that these cells contain all of the important steroid synthetic enzymes except CYP17, whereas oocytes contain CYP17 but no other relevant activities. It is still possible that our oocyte preparations contain a small population of follicular cells that contain all of the detected CYP17 activity; however, this explanation seems less likely for the following reasons. First, this population of cells would have to be so tightly associated with oocytes that it was completely resistant to separation by both collagenase and trypsin, as the follicular cell preparations contained no detectable CYP17. Second, this contaminating population of cells would have to be very small, as it was undetectable by both stereoscopic and histologic examination. Third, in order for such a small contamination to be mediating the high velocities recorded in Table I, which are very similar to the velocities of other CYP17 enzymes overexpressed in fibroblast or COS cells, as well as with those seen in cultured thecal cells (45–47), the turnover rate of the Xenopus CYP17 would have to be extremely high. We know that this is not the case, as comparison of the human and Xenopus CYP17 17α-hydroxylase activities in HEK cells (Fig. 5), and recently in yeast microsomes (data not shown), reveals that they have nearly identical turnover rates (~6 min$^{-1}$). Finally, progesterone injected directly into oocytes is immediately converted to AD (8), arguing that the enzymatic activity is within the oocyte itself.

We are left with the intriguing notion that, in the frog ovary, germ cells, or oocytes, play a critical role in the production of the steroid used for their own maturation. To our knowledge, this is the first example of germ cells being directly involved in steroid production, although it has not been carefully examined in other lower vertebrates, such as fish. Because oocytes make up >90% of the ovarian volume in frogs and fish, one could speculate that such lower vertebrate animals might require their oocytes to contribute to ovarian sex steroid production. In contrast, higher vertebrates, in which the ovarian volume primarily consists of follicular cells, may no longer need oocytes to subserve this function.

Interestingly, this concept of oocytes actively participating in their own maturation (for our case through the production of androgens) is consistent with the recently described work in mammalian systems, where oocytes have been shown to communicate with surrounding somatic cells to promote granulosa cell proliferation and differentiation (48). Oocytes may therefore utilize many different mechanisms to assist in orchestrating proper follicular development and subsequent ovulation.

Finally, these studies further explain and support earlier work suggesting that, although progesterone is capable of promoting maturation in vitro, androgens may be the primary mediators of maturation in vivo, where very little progesterone is ever produced. Steroid-induced maturation of Xenopus oocytes has been a puzzling field of investigation for many years, as it appears to occur independent of transcription and may involve signaling via classical steroid receptors acting outside of the nucleus (6–8, 49). Further studies of Xenopus oocyte maturation by androgens, in addition to progesterone, may aid in finally determining the details behind this complex and fascinating process.

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