Prostate-Specific Antigen Immunoreactivity in Amniotic Fluid

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We examined whether the 33-kDa serine protease prostate-specific antigen (PSA) is present in amniotic fluid and, if so, whether its concentration changes with gestational age. Analyzing 115 amniotic fluids with a highly sensitive immunofluorometric procedure, we found PSA in all the amniotic fluids examined and established that its concentration increases with increasing gestational age from 11 to 21 weeks, decreasing at delivery. PSA in amniotic fluid is present predominantly in the free (33 kDa) form; a minor fraction (<20%) is present bound to α1-antichymotrypsin. No significant correlation was seen between PSA and α-fetoprotein (AFP) in amniotic fluid or maternal serum in samples with high AFP. Amniotic fluid PSA was measurable by two different established methods for PSA. Pregnant women had higher concentrations of serum PSA than nonpregnant women. The highest PSA concentration in amniotic fluid was associated with a pregnancy that was complicated by the Rhesus incompatibility syndrome but the source of the PSA was not established. From recent literature reports, and the association of PSA with prostate and breast tumors, we think PSA may serve as a growth factor regulator in cancer and in normal fetal development during pregnancy.

Indexing Terms: fetal status/steroid hormones/hormone receptors/pregnancy-associated proteins

Prostate-specific antigen (PSA) was until recently considered a highly specific biochemical marker for prostate epithelial cells. PSA biochemistry and its clinical applications in screening, diagnosing, and monitoring prostate cancer have recently been reviewed (1, 2). We have also reported that PSA immunoreactivity can be detected in ~30% of breast tumors (3, 4) and demonstrated that breast cancer cells in culture can produce immunoreactive PSA after stimulation by steroid hormones (5). The immunoreactive PSA in breast tumors shares striking similarities with seminal PSA and is measurable by many commercial PSA kits. The presence of PSA in breast tumors is closely associated with the presence of steroid hormone receptors (4).

We hypothesized that PSA may also be present in biological fluids other than seminal plasma, male se-

rum, or tumor extracts. Here we report presence of immunoreactive PSA in amniotic fluid. The source and biological role, if any, of PSA in amniotic fluid need further investigation.

Materials and Methods

Methods. PSA determinations were carried out with an ultrasensitive time-resolved immunofluorometric assay, described in detail elsewhere (6). For comparative studies, we also used a commercially available automated PSA assay (IMx; Abbott Laboratories, Chicago, IL) (7). HPLC and Western blot analysis for PSA were performed essentially as described elsewhere (5, 6). Highly purified seminal plasma PSA, a gift from T. Stamey, Stanford University, was radioiodinated with [125I] by using Iodobeads (Pierce Chemical Co., Rockford, IL) according to the procedure recommended by the manufacturer. Most of the nonincorporated [125I] was removed by gel filtration on disposable PD-10 Sephadex columns (Pharmacia, Uppsala, Sweden). The LNCaP cell line was cultured as previously described (5).

Samples. The amniotic fluids analyzed were left over from routine amniotic fluid analyses for α-fetoprotein (AFP) or bilirubin (for diagnosis of neural tube defects or hemolytic syndromes, respectively) and were provided by L. Allen, Department of Clinical Biochemistry, Toronto General Division, The Toronto Hospital. The gender of the fetuses was not available at the time of the amniocentesis. Amniotic fluids at term, collected during labor, were provided by V. Davies, Toronto East General Hospital. The amniotic fluids were kept frozen at ~20°C until analysis. Cord blood was collected just after delivery into tubes without any anticoagulant. Cord blood serum and maternal serum were separated and stored at ~20°C until analysis (<3 weeks). Our study was approved by the Ethics Committee of the Toronto Hospital, Toronto, Canada.

Results

We initially analyzed some amniotic fluid samples for PSA with our ultrasensitive assay (6) and various combinations of assay buffers. This study showed that, to quantify the immunoreactive PSA (IR-PSA) in amniotic fluid, the assay buffer composition should be identical to that reported for serum samples, i.e., containing 60 g of bovine serum albumin (BSA), 0.5 mol of KCl, and 5 mL of Tween 20 per liter. If Tween 20 is omitted, the IR-PSA is underestimated, suggesting that PSA in amniotic fluid may be loosely bound to amniotic fluid components. Diluting the amniotic fluid can also eliminate this binding (Table 1). Recovery studies, performed with amniotic fluids with added
Table 1. Effect of assay buffer composition on immunoreactive PSA values in amniotic fluid.

<table>
<thead>
<tr>
<th>Buffer</th>
<th>Sample A&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Sample B</th>
<th>Sample C</th>
<th>Sample D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay buffer&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.00 (100)</td>
<td>2.17 (100)</td>
<td>0.21 (100)</td>
<td>0.26 (100)</td>
</tr>
<tr>
<td>BSA</td>
<td>5.45 (109)</td>
<td>1.48 (68)</td>
<td>0.059 (28)</td>
<td>0.047 (18)</td>
</tr>
<tr>
<td>BSA + KCl</td>
<td>6.00 (120)</td>
<td>1.48 (68)</td>
<td>0.055 (26)</td>
<td>0.062 (24)</td>
</tr>
<tr>
<td>BSA + NMS</td>
<td>4.95 (99)</td>
<td>1.30 (60)</td>
<td>0.086 (41)</td>
<td>0.088 (34)</td>
</tr>
<tr>
<td>BSA, 10 g/L</td>
<td>4.75 (95)</td>
<td>1.34 (62)</td>
<td>0.057 (27)</td>
<td>0.073 (28)</td>
</tr>
<tr>
<td>BSA + Tween 20</td>
<td>4.40 (88)</td>
<td>1.84 (85)</td>
<td>0.14 (67)</td>
<td>0.21 (61)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Results with assay buffer = 100%.
<sup>b</sup> Per liter, 60 g of BSA, 0.5 mol of KCl, 5 g of Tween 20, and 50 mL of normal mouse serum (NMS).
<sup>c</sup> Sample A contained 500 µg/L IR-PSA and was analyzed after 100-fold dilution in the respective buffer.

purified seminal plasma PSA, have also shown that recovery is incomplete (20–60%) if the assay buffer contains only BSA (data not shown). Recovery was almost complete (89% ± 14% and 101% ± 6%, respectively) when seminal plasma PSA or PSA from the serum of a prostate cancer patient was added to amniotic fluids and analyzed with the assay buffer described above (Table 2). Dilution linearity was checked by analyzing one serum sample and five amniotic fluids either undiluted or diluted as much as 32-fold. The data (Table 3) confirmed good linearity in all cases.

We analyzed 115 amniotic fluids for immunoreactive PSA with our ultrasensitive assay (6). For most of these samples, information was also available on amniotic fluid AFP values, gestational age, and maternal age. All amniotic fluid samples had detectable IR-PSA, between 0.012 and 16 µg/L, except for one with 500 µg/L. The 5th, 10th, 25th, 50th, 75th, and 90th percentiles for IR-PSA concentrations in these amniotic fluids were 0.039, 0.055, 0.13, 0.34, 0.93, and 2.11 µg/L, respectively. We found a weak negative correlation between IR-PSA and amniotic fluid AFP and no correlation between amniotic fluid IR-PSA and maternal age. There was a positive correlation between IR-PSA and gestational age, and we confirmed the known negative correlation between amniotic fluid AFP and gestational age after 15 weeks of gestation. A correlation between amniotic fluid AFP and maternal age was also observed. These correlation studies are summarized in Table 4.

Figure 1 shows a plot of the IR-PSA values and amniotic fluid AFP values in relation to the gestational age. Amniotic fluid AFP declines after the 15th gestational week, as previously reported (8), but IR-PSA tends to increase with gestational age. IR-PSA concentrations were very low before the gestational age of 12–13 weeks. Median values per gestational week are presented in Table 5.

We also analyzed 33 cord blood sera collected during delivery. From these, 31 had serum IR-PSA <0.05 µg/L; only two sera had greater values: 0.098 and 0.17 µg/L. Maternal sera at 15–20 weeks of gestation were also analyzed for IR-PSA. Of the 43 samples tested, 15 (35%) had IR-PSA ≥0.050 µg/L and 6 (14%) had ≥0.10

Table 2. Analytical recovery of PSA added to amniotic fluids.

<table>
<thead>
<tr>
<th>PSA, µg/L</th>
<th>Initially present</th>
<th>Added&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Recovered&lt;sup&gt;c&lt;/sup&gt;</th>
<th>% recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.30</td>
<td>6.48</td>
<td>4.62</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>1.82</td>
<td>6.48</td>
<td>5.45</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td>0.76</td>
<td>6.48</td>
<td>6.43</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>0.64</td>
<td>6.48</td>
<td>6.46</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>1.66</td>
<td>7.95&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.65</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>0.87</td>
<td>7.95&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.79</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td>0.68</td>
<td>7.95&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.59</td>
<td>108</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Added as seminal plasma PSA except as indicated otherwise.
<sup>b</sup> Added as serum of a prostate cancer patient.
<sup>c</sup> The recovered amount was calculated by subtracting the concentration initially present from the measured concentration after the addition.

Table 3. Dilution linearity of the PSA assay for amniotic fluids and serum.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>None 2 4 8 16 32</td>
</tr>
<tr>
<td>Amniotic fluid</td>
<td>A  B  C  D</td>
</tr>
<tr>
<td>A</td>
<td>ND&lt;sup&gt;d&lt;/sup&gt; 3.66 2.09 1.13 0.58 0.31</td>
</tr>
<tr>
<td>B</td>
<td>ND&lt;sup&gt;d&lt;/sup&gt; 8.29 5.40 3.00 1.45 0.74</td>
</tr>
<tr>
<td>C</td>
<td>6.07 3.70 2.60 1.40 0.78 0.42</td>
</tr>
<tr>
<td>D</td>
<td>2.44 1.38 0.80 0.40 0.21 0.12</td>
</tr>
</tbody>
</table>

<sup>a</sup> All PSA values are in µg/L.
<sup>b</sup> Not done (sample depletion).

Table 4. Results of linear correlation studies with 107 amniotic fluids.

<table>
<thead>
<tr>
<th>x</th>
<th>Linear regression</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>y = AF IR-PSA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>y = 2.46 - 0.091x</td>
<td>0.28</td>
<td>0.003</td>
</tr>
<tr>
<td>Gest. age, weeks</td>
<td>y = -4.92 + 0.39x</td>
<td>0.41</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mat. age, yrs.</td>
<td>y = 2.45 - 0.042x</td>
<td>-0.08</td>
<td>0.43</td>
</tr>
<tr>
<td>y = AF AFP&lt;sup&gt;d&lt;/sup&gt;</td>
<td>y = 4.22 - 1.68x</td>
<td>-0.58</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gest. age, weeks</td>
<td>y = 0.33 + 0.55x</td>
<td>0.33</td>
<td>0.001</td>
</tr>
</tbody>
</table>

<sup>a</sup> Amniotic fluid immunoreactive PSA in µg/L.
<sup>d</sup> Amniotic fluid AFP in mg/L.
μg/L, the maximum being 0.34 μg/L. In a previous study of 674 apparently healthy women (9), we found IR-PSA ≥0.050 μg/L in only 2% of women of all ages, as assayed with the same method (6), and IR-PSA ≥0.10 μg/L in 1% of the women (9). Values ≥0.10 μg/L were seen in only 1 of 572 women younger than age 50 (9).

We tested four amniotic fluids with AFP concentrations 3–21 times the median for the gestational age and four maternal sera with AFP 4–10 times the median for the gestational age. We saw no apparent relationship between amniotic fluid IR-PSA concentrations and AFP concentrations in patients with highly increased AFP, either in amniotic fluid or in maternal serum (Table 6).

We analyzed 19 amniotic fluids by our method and by the IMx PSA assay, a widely used automated assay commercially available from Abbott Laboratories (7).

The amniotic fluid sample with the highest IR-PSA immunoreactivity was also analyzed in dilution; it gave the result of 500 μg/L by our method and 440 μg/L by the IMx method. Results of the two methods correlate and agree very well (Fig. 2).

HPLC of amniotic fluids revealed that IR-PSA is present in at least two forms (Fig. 3). The major form (>80%) has a molecular mass identical to seminal plasma PSA (~33 kDa). A minor form (<20%), with a molecular mass of ~100 kDa, corresponds to PSA complexed with α1-antichymotrypsin (10–12). The identity of this peak was confirmed with an assay that specifically measures the PSA–antichymotrypsin complex (6, 10–12). In serum, this complex is the major form of PSA (6); free PSA is a minor fraction (Fig. 3B). Another PSA-containing complex of ~300 kDa was also seen at relatively low concentrations in some amniotic fluids, but its identity was not established (Fig. 3D).

![Amniotic Fluid PSA and AFP Concentrations](image)

**Fig. 1. Distribution of IR-PSA and AFP concentrations by gestational week in 107 amniotic fluids.**

The median values for completed gestational weeks are connected with a solid line. Only one sample was available at weeks 13 and 19.

<table>
<thead>
<tr>
<th>Gestation, weeks</th>
<th>No. of samples</th>
<th>Median IR-PSA, μg/L</th>
<th>Median AFP, mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>5</td>
<td>0.022</td>
<td>19.1</td>
</tr>
<tr>
<td>12</td>
<td>13</td>
<td>0.059</td>
<td>20.5</td>
</tr>
<tr>
<td>14</td>
<td>16</td>
<td>1.27</td>
<td>19.7</td>
</tr>
<tr>
<td>15</td>
<td>38</td>
<td>0.32</td>
<td>18.6</td>
</tr>
<tr>
<td>16</td>
<td>18</td>
<td>0.32</td>
<td>15.7</td>
</tr>
<tr>
<td>17</td>
<td>9</td>
<td>0.75</td>
<td>9.3</td>
</tr>
<tr>
<td>18</td>
<td>4</td>
<td>3.18</td>
<td>7.8</td>
</tr>
<tr>
<td>21</td>
<td>3</td>
<td>2.40</td>
<td>4.5</td>
</tr>
<tr>
<td>Term (40 + 2)</td>
<td>6</td>
<td>0.13</td>
<td>—</td>
</tr>
</tbody>
</table>

**Table 5. Median values for IR-PSA and AFP in amniotic fluids per week of gestation.**

![Correlation of PSA Results](image)

**Fig. 2. Correlation of PSA results between our time-resolved immunofluorometric assay (TR-FIA) (6) and the commercially available IMx PSA assay (7) for 19 amniotic fluids.**

The regression equation is: TR-FIA = 1.06 IMx + 0.22 (r = 0.96).
To examine whether more PSA binders are present in amniotic fluid that could form immunologically non-measurable complexes with PSA, we radiiodinated seminal plasma PSA and examined its distribution after dilution in a Tris buffer (control), amniotic fluid, or serum. Radioactive PSA added to amniotic fluid or serum was incubated for at least 1 h before injection into the HPLC gel-filtration column. Under the conditions of this experiment, free radiiodinated PSA eluted at fraction 62, corresponding to a molecular mass of 33 kDa (Fig. 4). In amniotic fluid, a minor PSA-containing component eluted at fraction 50 (molecular mass, 100 kDa), corresponding to PSA bound to α1-antichymotrypsin. In serum, in addition to free and α1-antichymotrypsin-bound PSA, another PSA-containing component eluted at fraction 34, corresponding to a molecular mass of ~700 kDa; this complex represents PSA bound to α2-macroglobulin (10–12), a complex not measurable by immunological assays for PSA. These data, and those of Fig. 3, confirm that amniotic fluid PSA is present predominantly in its free, 33-kDa form. A minor fraction exists as a PSA–antichymotrypsin complex. The α2-macroglobulin–PSA complex does not seem to exist in appreciable amounts in amniotic fluid, presumably because of the absence of α2-macroglobulin in this fluid.

Western blot analysis of amniotic fluid with a rabbit polyclonal anti-PSA antibody showed that amniotic fluid PSA appears at positions identical to those of seminal PSA or PSA produced by the prostate cancer cell line LNCaP (Fig. 5). Amniotic fluids also contain another immunoreactive band appearing at ~25 kDa and one at >200 kDa. The 25-kDa band was also found in extracts from normal breasts but not in extracts from breast tumors (data not shown). We did not identify the 25-kDa band, although it clearly accumulates in amniotic fluid with increasing gestational age (Fig. 5).

The high-M, and 25-kDa bands detected by Western blot analysis in amniotic fluid were not measurable by our highly specific and sensitive PSA immunofluorometric assay: Many amniotic fluids with prominent 25-kDa bands had very low PSA concentrations as measured by the immunofluorometric assay. Additionally, no peaks with molecular masses other than 100 or 33 kDa were detected in our HPLC studies (Figs. 3 and 4). These bands apparently represent proteins that cross-react with the polyclonal anti-PSA antibody but not with our capture monoclonal anti-PSA antibody. Alternatively, the high-M, bands may correspond to nonimmunoreactive PSA complexes and the 25-kDa bands to fragmented PSA, or to PSA produced by alternative mRNA splicing.

We additionally examined whether the 25-kDa band could originate from PSA fragmentation during β-mercaptoethanol reduction in the Western blot procedure, as is known to happen with purified seminal PSA. Purified seminal PSA or PSA from diluted semen generate fragments of ~21, 17, and 12 kDa, which are distinct from the 25-kDa band seen on Western blots (see Fig. 5). This confirms that the 25-kDa band is not simply an artifact of the reduction step in Western blots.

To examine further the stability of PSA in amniotic fluid at 37°C, we added to two amniotic fluids seminal PSA or PSA in a highly positive amniotic fluid. In one amniotic fluid, PSA was stable at 37°C for 5 days; in the other, PSA was degraded, generating a 16-kDa band (data not shown).

The amniotic fluid with the extremely high concen-
Fig. 4. HPLC separation of radioiodinated seminal plasma PSA diluted in Tris buffer (A), in amniotic fluid (B), or in human serum (C).

Each fraction was counted for radioactivity (counts per minute). The flow rate was 0.3 mL/min. The column was calibrated with molecular mass standards eluting at fraction 34 (660 kDa), 46 (160 kDa), 57 (44 kDa), 65 (17 kDa), and 76 (1.4 kDa); free $^{125}$I elutes at fraction 80. PSA, ACT-PSA, and A2M-PSA refer to fractions of free PSA or PSA bound to $\alpha_1$-antichymotrypsin or $\alpha_2$-macroglobulin, respectively.

Table 7. PSA concentrations in amniotic fluid at term.

<table>
<thead>
<tr>
<th>Patient</th>
<th>PSA, $\mu$g/L</th>
<th>Abstinence from sex, weeks$^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.51</td>
<td>20</td>
</tr>
<tr>
<td>B</td>
<td>0.099</td>
<td>4</td>
</tr>
<tr>
<td>C</td>
<td>0.13</td>
<td>4</td>
</tr>
<tr>
<td>D</td>
<td>0.086</td>
<td>1</td>
</tr>
<tr>
<td>E</td>
<td>0.059</td>
<td>1</td>
</tr>
<tr>
<td>F</td>
<td>1.04</td>
<td>4</td>
</tr>
</tbody>
</table>

$^*$ Weeks before delivery; all were physiological deliveries at 40 + 2 weeks of gestation.

Fig. 5. Western blot analysis of PSA-containing samples.

The amount of PSA loaded per lane is listed in parentheses: lane 1, molecular mass markers; lane 2, purified seminal PSA (6 ng) dissolved in 10 g/L BSA and showing a prominent 33-kDa band (overexposed) and bands at 21, 17, and 12 kDa; lane 3, PSA-containing supernate from the LNCaP prostatic carcinoma cell line (0.6 ng), showing a single 33-kDa band; lanes 4 and 5, highly PSA-positive amniotic fluid (500 $\mu$L) diluted 2-fold (2.5 ng) and 10-fold (0.5 ng), respectively, showing two prominent bands at 33-kDa (PSA) and 25-kDa (identity unknown); lanes 6–11. Amniotic fluid at 11, 12, 13, 15, 16, and 27 weeks gestation, respectively (<20 pg, which is below the detection limit of the Western blot). The amniotic fluids in lanes 6–11 had IR-PSA < 2 $\mu$g/L; notice the accumulation of the 25-kDa band with increasing gestational age.

Discussion

Until very recently, PSA was considered one of the most specific biochemical markers and thought to be produced exclusively by the epithelial cells of the prostate (1, 2). We have shown that PSA is produced by ~30% of breast tumors and provided evidence that PSA production is mediated by steroid hormone receptors, predominantly the progesterone receptor (3, 4). PSA, therefore, may also be a new favorable prognostic indicator in breast cancer.

We hypothesize that the PSA gene may also be expressed in situations associated with steroid hormone overproduction, such as during pregnancy, when the placenta produces massive amounts of steroid hormones (8). Moreover, there is now evidence that PSA may have biological functions other than the postulated seminal liquefication (1, 2). For example, Cohen et al. (13) found that PSA, a serine protease, cleaves insulin-like growth factor binding protein-3 (IGFBP-3), which is the major binding protein of insulin-like growth factor I (IGF-I) in serum. This finding suggests that PSA may modulate IGF function by altering the IGF–IGFBP-3 interactions. Kanety et al. (14) have also shown that serum PSA concentrations in prostate cancer are positively correlated with insulin-like growth factor-binding protein-2 (IGFBP-2) and nega-
tively correlated with levels of IGFBP-3. Killian et al. (15) recently found that low concentrations of PSA act as mitogens for osteoblast cells, an effect that may be due to activation by PSA of latent human transforming growth factor β. The same authors also found proteolytic modulation of cell adhesion receptors by PSA. España et al. (16) showed that PSA forms complexes with protein C inhibitor in semen and demonstrated the presence of this inhibitor in amniotic fluids. However, no report has as yet been published examining the presence of PSA in amniotic fluids.

Here, we have clearly shown that PSA immunoreactivity could be detected in all amniotic fluids tested. PSA concentrations, being very low at gestational ages of 11–13 weeks, increase as pregnancy progresses to 21 weeks. At term, the amniotic fluid PSA concentration returns to very low values (Table 5).

We had no access to fetal serum during pregnancy but our analysis of PSA in fetal cord blood serum at term showed that PSA concentrations are very low in most of them. Only 2 of 33 cord blood sera had PSA >0.05 µg/L. However, serum from pregnant women at gestational ages of 15–20 weeks had significantly higher PSA contents than nonpregnant women under age 50. In an extensive study of PSA presence in 674 sera from normal women, the overall prevalence of PSA concentrations ≥0.050 µg/L was 1.6% (9). However, this rate dropped to 0.9% for normal women under age 50. In the present study, PSA contents ≥0.050 µg/L were found in sera from 35% of the pregnant women. The source of PSA in the serum of pregnant women is unknown but could result from either diffusion of PSA from amniotic fluid or production by the perirectal glands (17–19) or the breasts after stimulation by steroids produced by the placenta. Recently, we found that normal human breasts can produce PSA after stimulation with steroid hormones (20). We have also shown that normal breast produces PSA and secretes it into the milk during lactation, postpregnancy (21).

We found no apparent relationship between PSA and AFP concentrations in amniotic fluid in patients who have highly increased AFP values in amniotic fluid (Table 6). The amniotic fluid PSA is predominantly in a free form; only a minor fraction is bound to α₁-antichymotrypsin, and probably no complexes between PSA and α₁-macroglobulin exist in amniotic fluid. We also present evidence that PSA could be proteolytically destroyed in at least some amniotic fluids. The amniotic fluid containing the highest concentration of PSA was associated with a fetus who developed neonatal hyperbilirubinemia due to Rhesus incompatibility syndrome. The relationship, if any, between amniotic fluid PSA and this hemolytic syndrome is currently unknown.

We do not as yet know the source of PSA in amniotic fluid. One possibility is that PSA enters the amniotic fluid by diffusion of sperm, which is very rich in PSA, through sexual contact during pregnancy. However, we found no apparent correlation between amniotic fluid PSA and length of abstinence from sex during pregnancy. At term, two patients with only 1 week of sexual abstinence before delivery had relatively low PSA concentrations and one patient with 20 weeks’ abstinence had concentrations 6- to 10-fold higher (Table 7).

This evidence now suggests that PSA may have important, previously unrecognized biological functions, including growth factor regulation (13–15). The appearance of PSA in breast tumors (3, 4) mediated through the action of the steroid hormone receptors (5) further suggests that the PSA gene may be expressed in cells other than those of prostatic epithelium and may play a role as a growth factor or a growth factor regulator. Subsequent to our reports of PSA production by breast tumors (3, 4) and by normal breast tissue (20, 21), Clements and Mukhtar have shown that PSA could also be produced by normal endometrium, another steroid hormone-responsive tissue (22). We speculate that the appearance of PSA in amniotic fluid, especially at relatively high concentrations near 14–21 weeks, may indicate a biological role of this protein in fetal growth and development.

PSA is a serine protease exhibiting extensive sequence homology with the human glandular kallikrein family of proteins (1, 2). Watt et al. have shown that PSA also shows extensive sequence homology with γ-nerve growth factor, epidermal growth factor binding protein, and α-nerve growth factor (23). Recent work by our group (20) has confirmed that the immunoreactive species measured by our assay is indeed PSA and not a PSA homolog. PSA mRNA was extracted from PSA-positive breast tissue and tumors, amplified by the polymerase chain reaction, and sequenced. The sequence homology between PSA mRNA obtained from the breast tissue and tumors and the PSA mRNA extracted from prostatic tissue was 100% (20).

We anticipate that this first demonstration of presence of a prostatic protein in amniotic fluid will initiate more studies examining the source, biological role, and diagnostic applications of this molecule during pregnancy.

We thank L. Allen and V. Davies for providing amniotic fluids and P.Y. Wong for PSA analysis by the IMx method. This work was supported by a grant from the Cancer Research Society Inc., Montreal, Canada, and the Ontario Chapter of the Canadian Breast Cancer Foundation. We also thank Leila Judithir for preparation of the manuscript.

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