Testing During Pregnancy

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Disclosures

• Abbott Point of Care, Inc.
  – Honorarium/Expenses
Objectives

• Explain the clinical utility of hCG testing in the diagnosis and management of intrauterine and ectopic pregnancy

• Compare and contrast recommendations for screening and diagnosing gestational diabetes mellitus

• Describe how thyroid function tests are affected by pregnancy
Human Pregnancy

- Approximately 40 weeks from 1st day of last normal menstrual period to expected day of confinement (delivery)
- Divided into 3 trimesters of ~13 weeks
- Term is considered 37-42 weeks
## Expected Physiological Changes

<table>
<thead>
<tr>
<th>System</th>
<th>Changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Circulatory</td>
<td>Increased blood volume (~45%)</td>
</tr>
<tr>
<td>Cardiac</td>
<td>Increased output (30-40%)</td>
</tr>
<tr>
<td>Renal</td>
<td>Increased GFR</td>
</tr>
<tr>
<td>Hepatic</td>
<td>Increased synthesis of plasma proteins</td>
</tr>
<tr>
<td>Endocrine</td>
<td>Insulin resistance/glucose intolerance</td>
</tr>
<tr>
<td>Respiratory</td>
<td>Hyperventilation and mild respiratory alkalosis</td>
</tr>
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Laboratory Management of the Pregnant Patient

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Human Chorionic Gonadotropin

PREGNANCY DIAGNOSIS
ECTOPIC PREGNANCY ASSESSMENT
Human Chorionic Gonadotropin (hCG)

- Glycoprotein hormone family

- hCG
- LH
- FSH
- TSH
Human Chorionic Gonadotropin (hCG)

- Glycoprotein hormone family

Adapted from *Trends Biochem Sci* 2004; 29:119-126
hCG Synthesis

• Synthesized by syncytiotrophoblasts

• Extends functional life of corpus luteum
  – Maintains increasing progesterone

• Serum concentrations increase progressively in early pregnancy
  – Peak at 7 – 9 weeks of gestation

• Decrease until ~24 weeks then plateau

Adapted from Expert Rev Mol Diag 2009;9:721-747
hCG Heterogeneity

- Numerous molecular forms of hCG present in pregnancy serum
  - Dissociated or degraded molecules with no biological activity

- Key β-containing variants
  - Intact hCG
  - Nicked hCG
  - Free β subunit
  - Nicked free β subunit
  - β-core fragment (urine)

Adapted from *Clin Chem* 1997;43:2233-2243
hCG in Normal Pregnancy

- Pregnancy diagnosis involves history & physical exam in conjunction with hCG testing

- Serum hCG detectable 9-11 days after LH surge
  - ~3-5 days before expected menses

- Urine hCG detectable around same time or soon after
  - More variable than serum

Adapted from *Fertil Steril* 2005;83:1000-1111
How early can hCG detect pregnancy?

- Depends on several variables
  - Length of menstrual cycle
  - Time from ovulation to fertilization
  - Time from fertilization to implantation
  - How expected day of menses is determined
    - Average cycle length
    - Days relative to LH surge or LH peak
How early can urine hCG detect pregnancy?

- Method used to determine day of menses influences timing of pregnancy detection
- As reference point, LH surge shows less variability for pregnancy detection

Adapted from *Curr Med Res Opin* 2009;25:741-748
Ectopic Pregnancy

• Extrauterine implantation of blastocyst
  – ~95% occur in fallopian tube

• Incidence is estimated at 2% of all pregnancies

• Responsible for 5% of maternal deaths

• Classic symptoms include abdominal/pelvic pain (95%) and vaginal bleeding (70%) but some have no symptoms until rupture

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Diagnosis of Ectopic Pregnancy

• Serial hCG
  – Prolonged doubling time in ectopic pregnancy
  – <53% increase in hCG over 48 h is 99% specific

• Transvaginal ultrasonography
  – Gestational sac should be evident at ≥42 days after conception

• hCG discriminatory zone
  – Surrogate marker for gestational age
  – Concentration above which, if no IUP visualized by US, a healthy singleton gestation is not present
  – 1,000 – 3,000 IU/L
Symptoms and + urine hCG → TVS

- IUP
- Non-diagnostic
  - >1500 IU/L
  - Serum hCG → hCG doubles
  - Repeat hCG in 48 h
  - hCG decreases → Failed pregnancy (IUP or ectopic)

- Ectopic
  - <1500 IU/L
  - Weekly hCG until negative

Reliability of the Discriminatory Zone

- 202 women who met the following
  - Transvaginal sonogram with no evidence of an IUP
  - Serum hCG measured on same day as sonogram
  - Subsequent documentation of a viable IUP

- hCG concentrations (IU/L)
  - 80% <1,000
  - 9% 1000-1,499
  - 6% 1,5000-1,999
  - 5% 2,000+

- Discriminatory zone should not be used to determine the management of a hemodynamically stable patient with suspected ectopic pregnancy
  - Follow-up sonography and serial hCG recommended

Table 1. Study Cases With β-hCG Above 2000 mIU/mL

<table>
<thead>
<tr>
<th>β-hCG, mIU/mL</th>
<th>Last Available Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>2215</td>
<td>Term liveborn singleton</td>
</tr>
<tr>
<td>2217</td>
<td>Term liveborn dichorionic diamniotic twins</td>
</tr>
<tr>
<td>2374</td>
<td>Term liveborn singleton</td>
</tr>
<tr>
<td>2530</td>
<td>Live singleton on 21-wk scan, then developed chorioamnionitis leading to pregnancy loss</td>
</tr>
<tr>
<td>2539</td>
<td>Term liveborn singleton</td>
</tr>
<tr>
<td>2993</td>
<td>Term liveborn singleton</td>
</tr>
<tr>
<td>4336</td>
<td>Term liveborn singleton</td>
</tr>
<tr>
<td>4476</td>
<td>Alive with large subchorionic hematoma on 7.6-wk scan, went on to first-trimester pregnancy loss</td>
</tr>
<tr>
<td>6567</td>
<td>Alive with slow heartbeat on 7.1-wk scan, then demise on 8-wk scan</td>
</tr>
</tbody>
</table>

*Drawn on the same day as sonography that showed no intrauterine fluid collection.*

*J Ultrasound Med 2011;30:1637-1642*
Oral Glucose Tolerance Tests

GESTATIONAL DIABETES MELLITUS
Gestational Diabetes Mellitus (GDM)

• Most frequent metabolic complication of pregnancy

• Any degree of glucose intolerance with onset or first recognition during pregnancy that is not overt diabetes

• Accounts for 90% of diabetes in pregnancy

• Affects ~7% of all pregnancies (range 1-14%)
  – Highest in ethnic groups with high frequencies of type 2 diabetes (Hispanic, African, Native America, Asian, and Pacific Island ancestry)
Pathophysiology of GDM

**Mother**
- Insulin availability (resistance)
- Glucose

**Placenta**
- Anti-insulin hormones:
  - Human placental lactogen
  - Estrogens
  - Progesterone

**Fetus**
- Pancreas
- Insulin
- Excess nutrient storage
- Macrosomia
- Hypoglycemia
Consequences of GDM

**Maternal Morbidity**
- Hypertension
- Preeclampsia
- Increased likelihood of C-section
- Development of diabetes after pregnancy

**Fetal Morbidity**
- Macrosomia (excessive birth weight)
- Neonatal hypoglycemia
- Polycythemia
- Increased perinatal mortality
- Congenital malformation
- Hyperbilirubinemia
- Respiratory distress syndrome
- Hypocalcemia
Testing for GDM

• Oral glucose tolerance test
  – Imprecise with fair reproducibility (~75%)

• Several testing protocols used world wide
  – Impossible to compare different studies of GDM
  – What is true prevalence of GDM?

• ACOG vs. ADA
# ACOG vs. ADA

<table>
<thead>
<tr>
<th></th>
<th>ACOG</th>
<th>ADA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Screening Test</strong></td>
<td>• 50 g glucose (non-fasting)</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>• 1 h glucose result exceeds:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- 130 mg/dL (85% sensitive)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- 140 mg/dL (90% sensitive)</td>
<td></td>
</tr>
<tr>
<td><strong>Diagnostic Test</strong></td>
<td>• 100 g glucose (fasting)</td>
<td>• 75 g glucose (fasting)</td>
</tr>
<tr>
<td></td>
<td>• 2 or more glucose results exceed cutoffs:</td>
<td>• 1 or more glucose results exceed cutoff:</td>
</tr>
<tr>
<td></td>
<td>- Fasting: ≥95 mg/dL</td>
<td>- Fasting: ≥92 mg/dL</td>
</tr>
<tr>
<td></td>
<td>- 1 h: ≥180 mg/dL</td>
<td>- 1 h: ≥180 mg/dL</td>
</tr>
<tr>
<td></td>
<td>- 2 h: ≥155 mg/dL</td>
<td>- 2 h: ≥153 mg/dL</td>
</tr>
<tr>
<td></td>
<td>- 3 h: ≥140 mg/dL</td>
<td></td>
</tr>
<tr>
<td><strong>Pregnancies diagnosed with GDM</strong></td>
<td>• 4 – 7%</td>
<td>• ~18%</td>
</tr>
</tbody>
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\[ \text{mg/dL} \times 0.0555 = \text{mmol/L} \]
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<th><strong>ADA</strong></th>
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<tr>
<td>- Originally established in 1964 (O’Sullivan &amp; Mahan)</td>
<td>- Adopted recommendations of International Association of Diabetes in Pregnancy Study Groups (IADPSG) in 2011</td>
</tr>
</tbody>
</table>
| - Cutoffs calculated as 2 SD of the mean whole blood glucose for each time point  
  - Predicted increased risk of diabetes after pregnancy | - IADPSG established new diagnostic criteria for GDM based on data from HAPO study |
| - Required 2 abnormal results to avoid “misclassification due to laboratory error” | - HAPO showed strong, continuous associations between maternal glucose and adverse outcomes |
| - Current cutoffs are adaptations of originals | |
Objective: to clarify the risks of adverse outcomes associated with various degrees of maternal glucose intolerance less severe than that in overt diabetes mellitus

23,316 pregnant women in 9 countries
  – 75 gram 2 hour OGTT
IADPSG Cutoffs

- Glucose cutoffs were those at which the odds for three specific HAPO outcomes were increased 1.75 times greater than mean HAPO concentrations (reference)
  - Birth weight >90th percentile
  - Cord C-peptide >90th percentile
  - Percent body fat >90th percentile

<table>
<thead>
<tr>
<th>Time relative to 75 g OGTT</th>
<th>Glucose (mg/dL)</th>
<th>Above cutoff (%)</th>
<th>Above cutoff (cumulative %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting</td>
<td>92</td>
<td>8.3</td>
<td>8.3</td>
</tr>
<tr>
<td>1 hour</td>
<td>180</td>
<td>5.7</td>
<td>14.0</td>
</tr>
<tr>
<td>2 hour</td>
<td>153</td>
<td>2.1</td>
<td>16.1</td>
</tr>
</tbody>
</table>

mg/dL x 0.0555 = mmol/L

_Diabetes Care_ 2010;33:676-682
# IADPSG GDM Detection Strategy

**Fasting glucose, random glucose, or Hb A1c at 1st prenatal visit**

<table>
<thead>
<tr>
<th>Fasting ≥126 mg/dL or Random ≥200 mg/dL or HbA1c ≥6.5%</th>
<th>Fasting 92 - 126 mg/dL</th>
<th>Fasting &lt;92 mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Overt diabetes</strong></td>
<td><strong>GDM</strong></td>
<td><strong>75 g 2 hour OGTT at 24-28 weeks</strong></td>
</tr>
</tbody>
</table>

mg/dL x 0.0555 = mmol/L

*Diabetes Care* 2010;33:676-682
Screening and Diagnosis of Gestational Diabetes Mellitus

**ABSTRACT:** *Gestational diabetes mellitus* (GDM), defined as carbohydrate intolerance that begins or is first recognized during pregnancy, is associated with increased maternal, fetal, and neonatal risks. The prevalence of GDM in the United States is increasing, probably because of increasing rates of overweight and obesity. A universal recommendation for the ideal approach for screening and diagnosis of GDM remains elusive. At this time, the Committee on Obstetric Practice continues to recommend a two-step approach to screening and diagnosis. All pregnant women should be screened for GDM, whether by patient history, clinical risk factors, or a 50-g, 1-hour glucose challenge test at 24–28 weeks of gestation. The diagnosis of GDM can be made based on the result of the 100-g, 3-hour oral glucose tolerance test, for which there is evidence that treatment improves outcome.

1. IADPSG criteria more than doubles the incidence of GDM
2. No evidence its use would produce clinically significant improvements in maternal and neonatal outcomes
3. Would significantly increase in health care costs
Which Protocol to Offer?

• No consensus on which protocol is best

• Get input of physicians

• May find it necessary to offer both
The Thyroid & Pregnancy

• Pregnancy places large demands on thyroid gland
  – 10% increase in size (higher with iodine-deficiency)
  – 50% increase in hormone synthesis
  – 50% increase in iodine requirement

• High prevalence of thyroid disorders in women of child-bearing age
  – Revealed by stressors of pregnancy
Increased Thyroid-binding Globulin (TBG)

- Estrogen increases glycosylation of TBG
  - Prolonged TBG half-life (from 15 min to 3 days)

- Increased hepatic TBG synthesis

- Affinity for T4 and T3 unaltered

\[ \text{mg/L} \times 0.0185 = \mu\text{mol/L} \]

*Acta Endocrinol* 1982;100:504-511
Increased Total T4 & T3

- Increased TBD leads to increased total T4 and T3

![Graph showing peak concentration at ~20 weeks during pregnancy.](image)

*Acta Endocrinol* 1982;100:504-511

\[ \text{nmol/L} / 12.87 = \mu\text{g/dL} \]
hCG Stimulation of the Thyroid

- hCG has weak thyrotropic activity
- TSH lowest at peak hCG concentration (~10 weeks)
- TSH may be below the non-pregnant reference interval (0.35-5.5 mIU/L)

J Endocrinol Metab 1990;71:276-287
Decreased Serum Free T4

- Slight increase in 1st trimester due to hCG stimulation of thyroid

- Remainder of pregnancy marked by decreased fT4

- Due to increased TBG, increased iodine clearance, and overall increased demand for T4
Increased Serum Thyroglobulin

• Often increased during pregnancy, especially in later weeks

• Associated with increase in thyroid gland volume (mean 10%) but goiter is rare in U.S.
  – 5-15% of women at term

\[ \text{ng/mL} \times 1 = \mu\text{g/L} \]

A: 1st trimester
B: Late gestation
C: Post-partum

Normal <30 ng/mL

J Endocrinol Metab 1990;71:276
Increased Renal Iodine Clearance

• Increased GFR leads to increased iodine clearance

• Decreased circulating iodine produces compensatory loss of thyroidal iodine
  – Clinically significant in iodine deficient states

• Increased dietary requirements of iodine
  – American Thyroid Association & Endocrine Society: 250 μg/day
- hCG stimulates thyroid
- TSH decreases as hCG increases
- Increased hepatic synthesis of TBG
- Increased TBG increases TT4
- Increased iodine clearance and increased demand for T4 decreases fT4
## Thyroid Function Tests During Pregnancy

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<td>TSH</td>
<td>• Use trimester-specific reference intervals if available otherwise use:</td>
</tr>
<tr>
<td></td>
<td>- 0.1-2.5 mIU/L (1\textsuperscript{st} trimester)</td>
</tr>
<tr>
<td></td>
<td>- 0.2-3.0 mIU/L (2\textsuperscript{nd} trimester)</td>
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<tr>
<td></td>
<td>- 0.3-3.0 mIU/L (3\textsuperscript{rd} trimester)</td>
</tr>
<tr>
<td>TT4 &amp; TT3</td>
<td>• 1.5x non-pregnant reference intervals</td>
</tr>
<tr>
<td>fT4 &amp; fT3</td>
<td>• Best to use equilibrium dialysis/LC/MS/MS method</td>
</tr>
<tr>
<td></td>
<td>• Use method- and trimester-specific reference intervals</td>
</tr>
<tr>
<td></td>
<td>• Interpret low results with caution</td>
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*Thyroid* 2011;21:1081-1125  
*Thyroid* 2007;17:1159-1167
Summary

• hCG is an early marker of pregnancy but should not be solely relied in the evaluation of ectopic pregnancy

• Clear associations between maternal hyperglycemia and adverse outcomes but no consensus regarding optimal protocol for identifying gestational diabetes mellitus

• Several expected changes to thyroid status during pregnancy
Self-Assessment Questions

1. Use of a low hCG discriminatory zone will
   A. Increase the sensitivity for diagnosing an intrauterine gestation
   B. **Increase the sensitivity for diagnosing an ectopic gestation**
   C. Decrease the number of women diagnosed with an ectopic pregnancy
   D. Decrease the number of hCG tests performed per patient

2. According to criteria recommended by the IADPSG, a fasting plasma glucose concentration 92 – 125 mg/dL during pregnancy is consistent with:
   A. **Gestational diabetes mellitus**
   B. Normal glycemia
   C. Overt diabetes mellitus
   D. Type 1 diabetes mellitus
   E. Type 2 diabetes mellitus

3. TSH decreases and free T4 increases during the first trimester of pregnancy because:
   A. Estrogen stimulates hepatic synthesis of thyroxine-binding globulin
   B. **hCG stimulates TSH-receptors in the thyroid gland**
   C. Increased release of thyroxine from thyroglobulin
   D. Thyroid-stimulating antibodies are transiently present during the first trimester