The goal of any method used to do the pregnancy examination is to determine the pregnancy status with 100% accuracy and have no false positives, no false negatives, determine the pregnancy as early as possible, have the ability to age the conceptus, be able to determine the viability of the conceptus, and possibly determine the sex of the fetus and have the results immediately.

**Rectal palpation**

- Currently, rectal palpation is the easiest, fastest, cheapest method, most accurate method that meets most of our goals.
- Your goal in rectal palpation is to be 100% accurate at determining the pregnancy status 35 days post-breeding.
- You need to set your own standards though. If you cannot determine pregnancy until 45 days post-breeding, then use that as your cutoff.
- You may even try to determine if cows are not pregnant by palpation of CL2 and F20 and uterine tone at 18-21 days post-breeding. This would indicate that the cow has low progesterone and is coming back into heat at the expected time and therefore is not pregnant. Conversely, a corpus luteum on the ovary and no tone in the uterus 21 days post-breeding indicates high progesterone and the cow may be pregnant. It does not however guarantee that the cow is pregnant.

**The Golden Rules of Rectal Pregnancy Exam**

1. You must examine the entire tract before declaring the cow open.
2. You must find one of the positive signs of pregnancy before you call a cow pregnant.
3. Pregnancy examination must always be the first step in your examination. If you not sure, recheck the cow...maybe in a few minutes, maybe tomorrow.
4. The only positive signs of pregnancy in the cow are fetus, cotyledons/caruncles, amniotic vesicle, and fetal membrane slip:
   - **fetus - simple enough!**
   - **cotyledons/caruncles**

Placentomes (cotyledons on the placenta/caruncles on the uterus)
amniotic vesicle (AV)

Click here and right click on the first frame to see a video on AV and FMS palpation.

fetal membrane slip (FMS) (chorioallantois)

Click here and right click on the first frame to see a video on a dissected AV and FMS palpation.

Technique of pregnancy diagnosis by rectal palpation of a retractable uterus

RETRACT, RETRACT, RETRACT !!!!!

You must retract the uterus to fully and accurately.

First feel the uterus for asymmetry. At 35 days of pregnancy the pregnant horn will feel slightly larger.

Asymmetry of the horns. Quite a bit actually.
Note the CL3 on ovary ipsilateral to the pregnancy.

Second, feel for fluid in the larger horn. The fluid has a smooth velvety feel because the uterine wall thins during pregnancy. The fluid almost feels like a water balloon that is not totally full. You must systematically feel the uterus for the amniotic vesicle, the fetal membrane slip, or the fetus. (cotyledons will not appear until about 75 days). Although the CL3 is on the ipsilateral ovary 99.9 % of the time and it may help to identify the pregnant horn, it is not a positive sign of pregnancy. Always follow the golden rules of pregnancy diagnosis.
Technique for pregnancy diagnosis by rectal palpation of a non-retractable uterus

Try hard to retract the uterus and examine the entire tract. Try to slip anterior to the cervix and feel for a fetus or cotyledons/caruncles.

Other signs suggestive of pregnancy are fremitus, which is a hypertrophy of middle uterine artery. The middle uterine artery can be picked up in the broad ligament and moved around. There is a fluid turbulence that gives a 'buzz' feeling to the artery. You must rule out the external iliac.

The middle uterine artery is in the broad ligament and movable. Do not confuse with the iliac artery, which is not moveable.

If there is no corpus luteum present, the cow cannot be pregnant.

Estimation of stage of pregnancy by rectal palpation

Why do you need to estimate the stage of pregnancy? There may be lost records and you need to predict dry off dates. You may need to stage pregnancy if records are not kept or a bull runs with the herd. You may need to confirm AI dates, or an AI date may not match what you feel. You may be asked to estimate parturition dates for beef herds.

Technique:

Fluid is barely palpable at 28 days, although this is not a positive sign of pregnancy.

Amniotic Vesicle (this is not recommend for student palpation)

The AV can be palpated as long as 2 weeks after is crushed. This would be a case where you find a positive sign of pregnancy, but the cow is not pregnant.

<table>
<thead>
<tr>
<th>Days Gestation</th>
<th>Width in mm</th>
<th>Width in fingers</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td>7</td>
<td>1/2</td>
</tr>
<tr>
<td>42</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>48</td>
<td>35</td>
<td>2</td>
</tr>
<tr>
<td>52</td>
<td>55</td>
<td>3</td>
</tr>
<tr>
<td>58</td>
<td>75</td>
<td>4</td>
</tr>
<tr>
<td>62</td>
<td>90</td>
<td>4+</td>
</tr>
<tr>
<td>65</td>
<td>105</td>
<td>5</td>
</tr>
</tbody>
</table>

Fetal Membrane Slip (be gentle, as you can damage a pregnancy by rough palpation)
Bovine pregnancy

32 days thread 1 horn
45 small string 1horn
60 string 2 horns
>70 large string

Cotyledons (actually the placentome, which is the cotyledon/caruncle unit). You must rule out that you are palpating an ovary by feeling at least 3.
75 days pea size
100 dime
115 nickel
125 quarter
150 half dollar
> 150 variable

Fetus
The fetus descends out of reach from 3-7 months.
You can first feel the fetus at 55-60 days inside the AV.
To estimate an aborted fetus they are:
2 months mouse
3 months rat
4 months small cat
5 months large cat
6 months beagle dog

Fremitus
Fremitus is not a positive sign of pregnancy, but can help staging.
At 5 months the artery ipsilateral to the pregnancy has fremitus.
At 6 months both the artery ipsilateral and contralateral to the pregnancy have fremitus.
At 7 months both arteries are large with fremitus and the fetus is ascending.

Differentials in Pregnancy Diagnosis by rectal palpation

- Failure to retract and no positive sign of pregnancy found can be caused by:
  - Incompetence - this will diminish as your skills increase.
  - Failure to be complete
  - Adhesions
  - Pyometra
  - Segmental aplasia
  - Mummification
  - Maceration
  - Lymphosarcoma
  - Granulosa cell tumor

Signs of problems in pregnancy noted with rectal palpation

Excess uterine tone, crowded cotyledons, decreased fluid, relaxation of pelvic diaphragm, bloody discharge, wrinkled FMS, gestation you palpate is not equal to the breeding date you are given (this could just be an error in the records though).

Summary of Rectal Palpation for Pregnancy Diagnosis

Establish the time you are confident in diagnosing pregnancy by rectal palpation. Always be systematic in your examination. Observe the golden rules of pregnancy diagnosis by rectal palpation.

Ultrasound

- There are different types of machines available.
- The most commonly used machines today are B-mode real-time, meaning that they produce an acoustic image in real time.
- The usually range from 3.5 - 7.5 MHz,
  - With greater MHz you see more detail but have less depth penetration.
  - There is more depth penetration with lower MHz, but less detail. It is always a tradeoff.
  - These machines cost from $10-20,000.
External

- Older external machines marketed by Animark and sold to farmers were 3.5 MHz external M-mode machines.
- These machines claimed 97% accuracy, but in fact they had many false positives pregnancy diagnoses.
  - This was used to the company's advantage by checking beef herds, where almost all the cows were pregnant.
  - Since the machines called almost all the cows pregnant, and they in fact were pregnant, the numbers looked very good for the machine.
  - In my experience the machine was 0% accurate when demonstrated by the company salesman.
  - These M-mode machines cannot estimate stage of gestation.
  - In order to really test a machine like this you need to test on it on a herd were half the cows are pregnant. This would tell much better how many false positives and false negatives the machine has.

Intrarectal

- Ginther used a machine to diagnose pregnancy at 12-14 days post insemination, but the average was 28 days.
- This was in a research setting, not a clinical setting.
- He also found that of 8 pregnant, 2 cows had early embryonic deaths.
- Intrarectal ultrasound is hard to do at first, and slightly more time consuming than rectal palpation, but will soon be much more common.
- Most people that do this find that days 26-28 give about 100% accuracy and the fetal heartbeat can be seen at 21 days.
- The gestational age can be approximated and the fetal viability can usually be determined.
- Ultrasound allows sexing of the fetus to be done at approximately 55-60 days of gestation. You look for the genital tubercle. This technique takes some time to learn.
- Ultrasound has many potential advantages over rectal palpation, but at present the cost is prohibitive.
- Fetal sexing can be done at about 60 days of gestation.

Milk progesterone assay

- This test is based on the milk progesterone concentration 21-24 days post breeding.
- The milk progesterone parallels blood progesterone.
- It is important to know that this can only be used with known AI or breeding dates and the test cannot be done randomly in a herd.
- It is based on the fact that if progesterone is low 21 days postbreeding then there is no chance that the cow can be pregnant.
- Remember that a cow must have progesterone be pregnant. Therefore the test is 100% accurate at non-pregnancy diagnosis when the progesterone is low.

If Progesterone is high 21 days after breeding the cow 'should' be pregnant.
bovine pregnancy

- If Progesterone is low 21 days after breeding (or anytime) the cow cannot be pregnant.
- However, if the progesterone is high, the test is only 80% accurate at diagnosing cows pregnant.
  - This is because there may be mistakes and cows may be bred that are not actually in heat.
  - These cows would therefore have high progesterone 21 days later, yet not be pregnant.
  - Also cows with short and long cycles can cause misinterpretation of the test.

- Note the chart above. A false positive if breeding was not done at estrus. The progesterone 21 days later may be high and indicate a pregnant cow. The blue line indicates the 2 ng/ml threshold value for positive.
- At one time a 'cow side' test that cost $3-5/test was marketed. Some other tests have also been marketed.

A 'cowside' milk progesterone test that is no longer marketed.

**Protein B (bovine pregnancy-specific protein B) (bPSB, bTP-1)**

- Protein B is secreted by trophoblast cells (binucleate giant cells).
- It is a radioimmunoassay procedure, and therefore is time consuming and relatively expensive.
- There are false positives early in the post partum because it is present in the post partum period.
- The first beef study was based on data 30 days after bull removal.
  - They found 99% accuracy on non pregnant (3/102), 95% on pregnant (99/102) 30 days after bull removal.
  - Palpation found 98/102 pregnant and 4/102 open.
  - In the end 98 cows calved, which looks very good.....but this has only been published in a lay journal and the palpation ability was unknown.
  - Also the article did not state which 98 cows actually calved. Was it the cow that the test said were
A study done in a dairy is shown below: *The numbers refer to the accuracy of the diagnosis.*

<table>
<thead>
<tr>
<th>Day</th>
<th>Day 24</th>
<th>Day 26</th>
<th>Day 30-35</th>
</tr>
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<tbody>
<tr>
<td>PSPB pregnant</td>
<td>86%</td>
<td>87%</td>
<td>90%</td>
</tr>
<tr>
<td>PSPB open</td>
<td>71%</td>
<td>89%</td>
<td>100%</td>
</tr>
<tr>
<td>Palpation pregnant</td>
<td>not done</td>
<td>not done</td>
<td>99%</td>
</tr>
<tr>
<td>Palpation open</td>
<td>not done</td>
<td>not done</td>
<td>100%</td>
</tr>
</tbody>
</table>

These results show that PSPB is not much, if any, better than palpation. Since you need an RIA, there will be a time delay between samples and results. Also, you cannot age, sex, or determine fetal viability. Needless to say, this technique is not used by anyone I know of.

Click here to link to a web page that sells the kits.

Cost is $1.67/cow (plus you have to bleed them and mail the samples in and wait for results) - The site provides an interesting calculator to determine how much you can save using this over palpation. (note how much is credited for EED and inaccuracy by rectal palpation)

**Early Pregnancy Factor**

Check this link out to a potentially exciting method to determine bovine pregnancy.

- EPF - two components
  - EPF-A - Uterine tube
  - EPF-B - Ovary
  - Production requires signal from fertilized ovum (ovum factor) released under prolactin presence after sperm penetration.
  - Appears 4-6 hours
  - Disappears with fetal death
  - Non-detectable at 20 days in milk and 30 days in serum
  - Lateral flow dipstick test

  - 17 non bred
  - 18 Day 6 embryo flush cows
  - Test sensitivity - 86%
  - Specificity - 4%
  - Positive predictive value - 49%
  - Negative predictive value - 23%
  - False-positive - 96%
  - False-negative - 14%
  - Table

<table>
<thead>
<tr>
<th>Test</th>
<th>Preg</th>
<th>Non</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnant</td>
<td>16</td>
<td>16</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>49%</td>
<td>PPV</td>
<td></td>
</tr>
<tr>
<td>Non-Pregnant</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>23%</td>
<td>NPV</td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td>18</td>
<td>17</td>
<td>86%</td>
</tr>
<tr>
<td>Sens</td>
<td>49%</td>
<td>Spec</td>
<td></td>
</tr>
</tbody>
</table>

Conclude that the ECF test is an unreliable method for determining pregnancy status of dairy...