The Importance Of Heat Detection In Cows
PRACTICAL APPLICATIONS
INTRODUCTION

The sexual activity of the adult bovine female, non-pregnant, and in good health condition is cyclic. The cycle lasts for 20 to 21 days with extremes varying from 17 to 24 days, this for heifers and for cows. The return in heat and the ovarian activity after calving comes back after 2 to 3 weeks to 2 or 3 months after delivery. Many factors influence the return in heat: the body condition, milk production, breed, the number and date of calving, as well as the post-calving pathology (1, 2, 7, 9, 10, 11, 12, and 16). Thus a dairy cows average interval between calving and the first ovulation is 20 days (8), and more than 80% of the animals observed in heat for the first time after 60 days have in fact already cycled (8). Beef cows tend to be slower to return to a sexual activity because the environmental conditions usually are not favourable (8).

Today one needs a herd management system which ensures economic viability. Heat detection is paramount and therefore must be priority when attempting to improve the global reproduction performance of one’s herd. Apart from the oestrus’s physiological signs (organic or hormonal), 24 hours before the onset of heat, one can already detect common sign: decrease in appetite, less resting period decrease, restlessness, mooing and decrease in milk production. Closer to the actual, sexual behaviour signs appear: vulva swelling, licking, fake fights, mounting attempts, pressure the chin on the back of an other cow, and vaginal mucus. However the actual heat starts the only visual is the mounting acceptance. This reaction lasts on average, 18 to 24 hours for cows and 9 to 12 hours for heifers (11,12).

HEAT DETECTION

Heat detection affects herd fecundity and fertility and it is the first criteria involved in reproduction management (5). Thus it is an essential condition of any successful breeding program. In a herd where heat detection is well done, 75% of the cows must be seen in heat at least once in 60 days following calving. The non-observation of heat during the 2 months following calving reveals 2 types of problems: 1) the cow was not in heat (true anoestrus) or 2) the detection was poor (14). Consequently, in both cases, the cow will be “open” longer and will be able to reproduce only after; Heat detection

Exhaustive technical and scientific articles have been written about heat detection. Still one out of two breeders does not detect the heat and one out of every six times the AI technician is called when the cow is not in heat. (1, 2, 3, 8)
The first infertility reason in a herd is often the difficulty to detect the heat when it appears. This can be caused by biological problems (short oestrus, cow in high milk production level, heat appearing mostly at night between 18H00 and 06H00 (11)) or due to breeding practises (not enough time devoted for detection, “own” criteria used by the breeder, substantial increase of the size of the herd).

Heat detection depends of two factors: 1) the animal and its capacity to show heat signs; 2) the breeder’s surveillance capacity and experience.

Various studies show that it is almost impossible even for an experienced breeder, to detect more than 80% of the heat even though he would observe the herd 3 times a day for a period of at least 30 minutes each. However the above recommendations are usually not followed by the breeder (4, 5, and 11).

Heat detection becomes yet harder to master when you know that, on average 20 % of the cows have short heat cycle, silent heat or exhibit heat signs only at night (6,8,12).

The visual observation of heat behaviour is, in these cases, so difficult that the breeder usually uses other less obvious signs than mounting. This explains why 5 to 20 % of the inseminated females have a high progesterone level (8).

Table 1: Herd spread according to the proportion of female inseminated in luteal phase: progesterone concentration at the time of AI > 1 ng/ml – Reimers and Coll 1985.

<table>
<thead>
<tr>
<th>Percentage of cow in luteal phase at AI(*)</th>
<th>Herd numbers</th>
<th>Herd percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>283</td>
<td>60,6%</td>
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<tr>
<td>&lt;10</td>
<td>40</td>
<td>8,8%</td>
</tr>
<tr>
<td>10-20</td>
<td>102</td>
<td>21,8%</td>
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<tr>
<td>&gt;20</td>
<td>42</td>
<td>9,0%</td>
</tr>
<tr>
<td>Total</td>
<td>467</td>
<td>100%</td>
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</tbody>
</table>

(*) Intra-herd

To obtain a calf per year for each cow, we have two possibilities:

* improve the cow’s capacity to fecundate (fertility);
* try to inseminate each female once two months after calving.

Heat detection is the best mean to improve the reproduction performance.

**WHAT ARE THE METHODS USED FOR COW HEAT DETECTION?**

I - **Non visual methods:**

A) Genital tract clinical evaluation;
B) Intra vaginal pH measurement or vaginal mucus conductivity;
C) Progesterone dosage;
D) Pedometer;
E) Vaginal or milk temperature recording
II - Visual Methods:

Immobilisation reflex: observation 3 times a day (Ex.: 6-7a.m, noon-1p.m. and 6-7p.m.) or through video surveillance around the clock.
Note that a late evening surveillance by the breeder tends to improve reproduction performances drastically (15).

a) Detection help:
   * Blowing animal: castrated bull, androgenised cow;
   * Marking system: tight-rope marking (Chin-ball).

b) Mounting markers:
   * Positioning of difference articles around the sacrum area: *Kamar®;
   *Ink Tel Tail; *DEC™.

ELECTRONIC HEAT DETECTORS USED AS A TOOL FOR BOVINE REPRODUCTION SURVEILLANCE:

In bovine, the first sexual behaviour sign is mounting acceptance. Thus, the time devoted to this observation by the breeder is directly linked with the period between calving and the first return in heat.

The improved efficacy of electronic heat detection devices for bovine would allow:
1) An improvement of heat detection especially for short heat, silent heat and heats appearing during the night.
2) A decrease in the amount of mistakes due to the breeder’s consideration of others factors not generally linked to oestrus.
3) An improvement of fertility as well as a reduction of the calving- AI interval if the system informs about the true beginning of the heat. This will always be better than the usual twice a day control;

IMV Technologies developed a heat detection device based on electronic recording. It is the first visual, alarm electronic heat detector. It takes into account all the elements mentioned above.
CONCLUSION

Mastering fecundity is the key element in herd management and profitability. The fertility improvement is directly linked to the working conditions which allows the herdsman to improve heat detection and inseminate at the right time. The various techniques available today on the market must improve the herd observation and thus the reproductive performances.

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Estrus Synchronisation In Cattle
INTRODUCTION

The heat synchronisation consists of modifying the cycle’s length or inducing oestrus. It allows one to choose the day and the time of the artificial insemination as well as the calving period.

For cattle, there are many advantages to successfully synchronise the oestrus:
- It allows a group of cows in heat to be gathered on a given day in order to inseminate them all at the same time.
- It eases heat detection
- It allows a better scheduling of delivering of calves (better calving surveillance, decrease in birth mortality and more homogeneous calves)
- It allows a decrease in unproductive periods by controlling the post-partum sub oestrus, especially in dairy cows.
- It allows one to choose the milk production peak during a period when the milk income/market price is higher.

SYNCRONISATION METHODS

Hormonal treatments are used in every technique of oestrus synchronisation. Nevertheless an induction groups the cattle ovulation better. This could be induced by various breeding practises called «zoo-technical methods »:

1. **Male effect:** After a confinement period for the heifers or cows, the introduction of a bull or a castrated but androgenised male induces an increase in LH’s peak frequency and amplitude, i.e. ovulation and an increase in heat behaviour.
2. **Group effect:** Breeding heifers by lots induces an earlier puberty and oestrus gathering.
3. **Flushing:** In dairy cows, fertility depends on body fat before reproduction. Flushing consists on increasing the feed energetic level in order to compensate an insufficient feeding level or a bad body shape. Practically on cows without enough body fat (mark < at 2.5 on a scale of 5), more feed for three weeks before and after AI, increases the fertility and reduces the embryo mortality.

Keep in mind that none of the above mentioned techniques allows a real heat synchronisation but they can be used along with hormonal treatment.

HORMONAL TREATMENT FOR OESTRUS SYNCHRONISATION:

Two methods are available:

- Shortening the luteal phase through products which purpose is to suppress the corpus luteum (luteolysis). Use prostaglandin (PGF2α) or analogs through injection. This method applies only for female already cycled. The PGF2α has no effect on corpus luteum at early development stage (between day 0 and 5) thus a normal protocol is to inject it twice, 11 to 13 days apart.
Use of synthetic progestagens through the vagina (spiral Prid®, CIDR®) or through skin implant (Crestar®). The progestagen can be used on females who are cycled or not. They can be injected along with PMSG (400 to 600 IU according to the breed, season and age) and prostaglandin (PGF2α or analogs). At the beginning of the treatment oestrogen are injected in the muscle or through the vagina.

SYNCHRONISATION PROGRAMS

PROSTAGLANDINS:

1. Double injection at 11 to 13 days of interval with two AI’s at fixed time 72 and 96 h or one AI at 80 h after the second injection.

The basic objective of this program is to have a high % of the herd in diestrus at the time of the second injection. In a cycled, herd approximately two thirds of the animals should be in diestrus at any given time, hence the majority should respond to a single injection. With these cows this response i.e. luteolysis is followed by oestrus in about 2 to 5 days later. 11 to 13 days after the injection the cows that responded should be on day 6 to 9 of the cycle; the remaining would be on day 6 to 15. Most of the animals should have a functional CL, and a second injection at this time should induce a synchronised oestrus. This program is used most often when heat detection is impossible, limited or very inefficient. It can be an efficient program in beef herds and in replacement dairy heifer program. The herd heat detection is established either by limited heat detection or by rectal palpation of the ovaries.

Insemination based on heat detection is still the best way to achieve the highest possible conception rate; generally cows are inseminated somewhere between 72 and 96 h after injection and heifers inseminated up to 10 hours earlier than cows.

2. Day 1 rectal palpation and treatment if mature CL

This practical and useful management tool in programs for dairy replacement heifers in which heat detection is possible. AI should always be based on heat detection in this program because the synchronisation’s rate following a simple dose of prostaglandin is not high enough for fixed timed AI.
3. **Day 1 herd must be cycled, or palpate each animal and treat only cycled animals and practise AI on heat detection. Day 12 treat all animals not yet bred and practise AI on detection:**

This is a variation of program 1 but the drug cost could be reduced proportionately by heat detection if a smaller number of cows receive 2 injections.

4. **From Day 1 to 5 detected cows in oestrus and inseminated, Day 6 inject all animals not bred and detect the estrus and inseminate.**

With this program in a cycled herd over 80% of the cows will be bred in the 10-day period. This is an economical program (only 75 % of the cows receives an injection), requires no ovarian palpation and is a practical method for shortening the breeding season in herds in which AI is used.

**Remember:**
- **Prostaglandin must be used just on cycled and non-pregnant females**
- **Injection time**
  - Between Day 0 (estrus) and Day 5: Prostaglandin’s does not works
  - Between Day 5 and 9: Optimal period
  - Between Day 10 and 15: cows become in oestrus later than Day 5-9
  - Between Day 15 and 21: Prostaglandins do not work

**• PROGESTERONES:**

The progesterone’s devices (ear implants or vaginal devices) act as an «artificial CL» and therefore prevent LH surges and ovulation, CL formation and CL maintenance. When the device is removed 8-12 days later, the pituitary gland released from the inhibitory effects of progesterone and the animal returns in oestrus in 24 to 36 h.

1. **Put an ear implant and inject intramuscularly norgestomet + estradiol valerate at Day 0, remove it at Day 9 or 10 with one AI at 48 to 56h after:**

  [Diagram: Ear implant with injection and removal schedule]

  To increase the degree of synchrony a prostaglandin could be injected 48 before removing the ear implant; the estrus fertility will be increased too:
In non-cycling females a PMSG injection must be done when the ear implant is removed:

D 0 ear implant + estradiol  D 9-10 ear implant remove + PMSG inj. 400-600UI

\[ \downarrow \] \[ \downarrow \] \[ \uparrow \]

\[ \text{PGF}2\alpha \]

2. Vaginal devices (PRID® or CIDR®) are usually used with the same schedule that ear implants (with or without PGF2\alpha and/or PMSG). The vaginal device will stay into the vagina for 8-12 days:

D 0 Vaginal device  D 8-12 remove the vaginal device + PMSG

\[ \downarrow \] \[ \downarrow \] \[ \uparrow \]

\[ \text{PGF}2\alpha \] one or two days before to remove

\[ \text{NB: The} \] choice between an ear implant or a spiral depends of the farm facilities for hold the animal. To put an ear implant, you will need 2 people and a contention cage but for the spiral a contention corridor is enough. Nevertheless, even though with the implanting an ear device can be a source of disease transmission through blood, we would recommend from our own experience the use of ear implant.
MANAGEMENT CRITERIA FOR SUCCESSFUL PROGRAMS

The introduction of anoestrus cows or heifers in synchronisation programs is a major factor that contributes to program failure. The following factors should be considered in the management of controlled breeding programs in cattle:

- **Nutrition:** Cows that are fed less than the amount needed to maintain energy levels and hence, lose weight over winter are plagued with delayed onset of postcalving estrus, resulting in anoestrus at the beginning of the breeding season. Also inadequate postcalving energy intake results in low first service conception rates.

- **Season:** The best conditions are when the days are long enough (12 to 14 hours day light) and when the average temperatures are in a comfortable range for the animals.

- **Heifers:** The best results are obtained if heifers are 14-15 months of age and have reached at least 65% of expected adult weight (Holstein breed). To achieve this, the farmer should feed properly the heifer between birth and 6 month of age. Any growth delay at 6 months could result in fertility problem later on.

- **Postpartum interval:** Cows less than 50 days postpartum should be excluded from synchronisation programs. First and second calf heifers usually require a post-partum interval longer than cows (i.e. 60-70 days).

- **Calving:** The hygiene of the calving area as well as the farmer and veterinarian hygiene affects the results. Thus difficult calving are oftenly associated with retained placenta which might gives accumulation in the uterus and metritis

- **Herd health:** The general health of the herd should be evaluated before starting an oestrus synchronisation program.

- **AI technicians and semen:** Adequate and sufficient AI technology should be ensure, especially if large number of cows are to be bred over a short period of time. Facilities should be adequate and include protection from sun and wind. The quality of the semen should be checked.

- **Stress:** Stress can have an effect when it occurs in luteal phase. Indeed it inhibits the LH hormone secretion thus the ovulation and increases the embryonic mortality. Consequently during 3 weeks before and after AI, it is advised not to vaccine, de-horned, change the food or de-worm the animals.

- **Lodging:** Best results are obtained in well-spaced area which should allow free movement of the animals and a well lighted area. An effect

REMEMBER THAT ESTRUS SYNCRONISATION IS NOT A “FERTILITY DRUG” AND WILL NOT INCREASE FIRST CONCEPTION RATE IN A BAD MANAGEMENT HERD
Bovine Artificial Insemination Checklist
When a commercial AI program is initiated the main objectives and advantages are:

1) More rapid genetic gain through the widespread use of superior sires, which most breeders could not afford to own,
2) Control of diseases which are spread through natural service and are a serious problem in many herds,
3) Improvements of the safety aspect on the farms through the elimination of bull particularly dairy bulls, which frequently cause serious body injuries and even death.

All efforts to make AI successful using the proper collecting, handling and processing of semen are worthless if the final stages of the AI procedure are not properly carried out.

The AI is performed in relatively unhygienic surrounding; therefore, the responsibility is placed on the inseminator to be clean in every possible aspect. The following are especially worth noting.

1) Keep your Insemination kit clean and organised.

2) Identify cow(s) and check breeding records. Standing or true heat is the most reliable sign of heat or estrus. If using other heat signs, it is best to observe at least 3 secondary signs (mucus discharge, friendly with observers, loss of appetite). In case of repeat, note if the animal in question is a late repeat or is she an early or quick repeat.

3) Observe the body condition (cows gaining weight conceive better than cows in a weight loss condition) and any genital discharges (pus = infection or blood = too late to breed)

4) Wash yours hands.

5) Have sleeve, sheath, gun, scissors and towels ready **before thawing semen**.

6) Prepare the thawing device at 35 °C-37 °C and place it near the liquid nitrogen semen tank.
7) Be sure that the canister containing semen is well below the top of the tank neck.

8) Identify the semen from the desired bull.

9) Remove the straws using tweezers as quickly as possible (recommended time is between 3-5 seconds); thaw only the straws you will use within 10 to 15 minutes. NEVER ALLOW SEMEN TO REFREEZE

10) Shake straw to remove excess Nitrogen and quickly plunge into the thawing unit for not less than 30 seconds. Do not thaw more than one straw at any (one) time.
11) During the thawing period prewarm the gun by stroking it vigorously with a paper towel five or six times; During HOT WEATHER, before loading the straw, make sure the gun is not too hot as it will heat the semen.

12) After the thawing period remove the straw from the water and dry it completely. **WATER IS LETHAL TO SPERMATOZOA**

13) Check the sire identification and the sealed end of the straw before loading the gun.

14) It may happen that after thawing, an air bubble is located in the middle of the straw. In this case grasp the straw at the factory seal and shake it once or twice like a thermometer, in order to force the air bubble to move from the middle to the sealed end of the straw. This location of the air bubble is important so that when the straw is cut no semen is lost, as well as ensuring continual flow of semen during the AI.

15) Cut the end of the straw with a pair of scissors. Care must be taken so that the end of the straw is not crimped, thus preventing a secure fit into the inseminating sheath. After cutting, wipe the scissors with a clean paper towel.
16) Carefully place the straw into the insemination sheath; the cut end of the straw should be fit snugly in the adapter within the plastic sheath. Grasp the adapter with the thumb and forefinger while twisting the straw into the adapter. This will insure the proper seating of the straw inside the adapter.

17) Place the stainless inseminating gun over the straw and slide the gun between the sheath and the straw. Do not use a cold gun. Gently push the gun and the straw toward the end of the plastic sheath. To anchor the plastic sheath to the gun remember that IMV has two kinds of inseminating device;
   a) The spiral Insemination Gun for 0.50 and 0.25 ml straws (The IMV Combi-plug) a machined screw-threads IMV Ref.D330 .to be used use with the non split sheaths (D402)
   b) The French straw gun used with a « O » ring and the split sheath.

18) Remove the air bubble by pushing lightly on the plunger. Your gun is ready to use now.

19) A protective IMV sanitary sleeve (Ref. D 801) should be used to prevent the spread of disease such as Ureaplasma and Mycoplasma, when present, from the vulva through the cervix and into the uterus. Also useful in cows with vulva/vaginal damage from calving

20) Place the loaded gun into a glove behind your back and secure it inside your shirt
21) Restrain the animal you will inseminate but do not tie down or over excite; MOST COMMON ERRORS OCCUR IF THE ANIMAL TO BE BRED IS NOT RESTRAINED OR IS POORLY RESTRAINED FOR AI.

22) A gloved hand (lubricated) is introduced into the rectum of the cow and used to locate the cervix through the rectal wall.

23) Clean the vulva thoroughly prior to insertion of the breeding gun with a dry paper towel. Do these two or three times and if necessary, use a new towel each time.

24) Insert the insemination gun at a 30° angle to avoid the urethral opening. If you use the IMV sanitary sleeve do not forget to remove it by pulling the sleeve before entering the cervix.
25) Pass rod through cervix, check location (uterine body) with the tip of index finger and slowly deposit the semen. REMEMBER AI IS A TWO STEP PROCESS, FIRST GET THE GUN TO THE CERVIX, AND THEN PLACE THE CERVIX OVER THE GUN. BE GENTLE AND TAKE YOUR TIME.

26) Slowly remove the gun and check for infection, blood, and semen backflow inside sheath.

27) Massage uterus very gently.

28) Properly dispose of sheath, towels and glove. Make sure not to throw dirty gloves and AI sheaths on the floor of the barn or in the gutter.

29) Clean the gun with alcohol 70° and dry it.

30) Clean and disinfect yours boots before leaving the farm.

31) Wash your hands before leaving.

32) After the last call of the day, wash outside of your AI kit. Empty the water from your thawing bath and wipe it dry inside and outside.

33) It is good practice to remove all your equipment from your AI kit and clean it thoroughly once a week.
Calving And Its Effects On Reproductive Performance In Cattle
In the bovine, conditions which can lower fecundity rates include increased calving interval (late breeder) or innate low fertility (return in heat after AI). The risk factors during calving are trauma and infection of the genital tractus.

**Farmer assisted calving**

During assisted calving or manual delivery, major trauma can happen due to poor knowledge or impatience. *Serious trauma:* Vulva, cervix or uterus tearing. These are fairly rare incidents in cows that can however put the life of the animal in danger.

*Benign trauma:* These kinds of traumas are very frequent. They happen about 50% of the time during difficult calving (dystocia) or prolonged calving (over 3h30 mn after the first signs of calving were triggered: head turning, stamping, legs blows towards the belly, lifting of the tail or if > 2 hours when colic signs due to the alternate rising and laying of the animal).

**Calving hygiene**

After calving, the cow uterus is contaminated 90% of the time due to bacteria from the environment. However, animals in good health get rid of them at the first signs of the next heat cycle.

Nevertheless, in case of trauma, due to the lack of hygiene in the environment or the lack of attention of the farmer or veterinarian, a massive contamination can occur that the uterus will not be able to overcome: endometritis follows.

To avoid these problems, it is utmost importance to respect the animal’s instinct as well as giving the dam optimum calving condition.

Let the dam calve in a quiet and clean environment. Calving should be occur in pasture (not too far from the barn in order to guarantee the surveillance of the animal) or in the barn in a specially dedicated and arranged “calving area”. The optimum for a 40 milking cow herd is to have a square pen of around 15 sq.m (165 sq.ft). Above 20 sq.m (220 sq.ft) it becomes difficult to immobilize the animal. Natural light should be coming through a translucent roof for about 10% of the overall surface area on the floor. The rest should be through a double neon tube lighting for night interventions. The area should be ventilated and equipped with water. The floor should be natural earth with straw or concrete as long as it is non-slippery (18 mm wide grooves and 10 mm deep). A small area for pharmaceuticals and/or drugs with a trash should be also dedicated.
Intervention (rectally) should only take place after:
- 3h30mn after observations of the first colic signs (+ head returning, tail raising and leg blows to the belly)
- 1h30mn after observing alternating laying and getting up.

Good hygiene is paramount to any intervention:

- The hind quarters, the vulva and surrounding area and the underside of the tail should be cleaned and disinfected,
- Use plastics sleeves,
- Use a nonirritating lubricant.

In case of retained foetal membranes (over 12 hours after calving) DO NOT deliver manually. This will increase the chances of metritis. Treatment should be through injectable prostaglandins or possibly antibiotics (intra-uterine tablet or otherwise are ineffective). Wait until the cow delivers the membranes spontaneously. This can take up to 10 days.
Pregnancy Diagnosis In Cows
The accurate and early diagnosis of pregnancy in both dairy and beef herds is essential for the maintenance of high levels of reproductive efficiency. It is required for the early identification of fertility problems at both the individual animal and herd levels and for the achievement of planned seasons of calving and prescribed calving interval.

There is a variety of methods of pregnancy diagnosis in cattle. *Failure to show estrus* 3 weeks following AI is often assumed to be indicative of pregnancy. However, even if estrus detection is good, not all of these cows will be pregnant. On the other hand 7% of the pregnant cows will show some signs of estrus during the first 3 months of pregnancy. Sometimes AI in these animals may result in embryonic mortality or abortion.

Many farmers still rely on external indications, such as udder development in heifers or an increase in abdominal size in cows, but by the time these signs fail to appear the herd breeding programme can be seriously impaired.

The *rectal diagnosis* of pregnancy in the cow by an experienced veterinarian is the earliest and most widely method available. The Fig 1 correlated the rectal findings and the pregnancy age; i.e.: the presence of placentomes is a positive sign from 3 months to the end of the pregnancy in the cow.

<table>
<thead>
<tr>
<th>Membrane slip</th>
<th>Amniotic vesicle</th>
<th>Palpation of the foetus</th>
<th>Placentomes</th>
<th>Fremitus uterine artery pregnant horn</th>
<th>Fremitus uterine artery empty horn</th>
</tr>
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<tbody>
<tr>
<td>1 month</td>
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<td>1.5 month</td>
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Fig 1: Relationships of palpable findings and gestational age in cows

For early pregnancy diagnosis (< than 70 days) the findings are:
- Asymmetry and fluctuation of the uterine horns,
- The membrane slip,
- The palpation of the amniotic vesicle.
For later pregnancy (> 70 days) the findings are:
- The foetus palpation,
- The placentomes,
- The fremitus,
- The cervix localisation.

Cow uterus at 3 months pregnancy

The potential advantages of using **ultrasonography rectal examination** for pregnancy diagnosis are that the presence of the foetus can be detected earlier than by palpation per rectum and that direct physical manipulation of the gravid reproductive tract is unnecessary with ultrasound. Performed between day 27-33 following AI.

Ultrasonography offers the opportunity of determining embryo viability by viewing the heartbeat of the embryo. On the other hand between day 55 and 60, male and female foetuses can be differentiated by an experienced operator of the relative location of the genital tubercle (presumptive penis or clitoris).