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# Reproduction in dairy cattle II

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# Foreword

This guide on reproduction in dairy cattle is presented in two partner volumes:

**Book I**
Gives the basic facts of reproduction in dairy cattle. Facts which should be familiar to (modern) dairy farmers, dairy farm managers and extension workers dealing with dairy farming.

**Book II**
This part deals quite extensively with artificial insemination ‘at work level’ in a (sub)tropical environment.

It should be noted that in many places in the (sub)tropics ‘extensive’ dairy farming (as opposed to ‘modern’) may be the most common practice and in fact is the most economic way to keep cattle (see annex book I). The fact that this part pays so much attention to artificial insemination does not at all mean to say that it is a ‘must’ in dairy farming everywhere.

**Acknowledgement**
The text of both books has largely been adapted from texts originally prepared by Prof. Dr C.H.W. de Bois and Dr Uwland, formerly lecturers in the International Course on Dairy Cattle Husbandry of the International Agricultural Centre IAC in Wageningen, the Netherlands.

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# Contents

# 1 Introduction to artificial insemination (AI)

Artificial insemination (AI) in cattle is a breeding method which is practised worldwide. In some countries (almost) 100% of the dairy cows are bred by AI.

## 1.1 Possibilities and advantages of AI

- With AI the direct contact between males and females is avoided. For that reason AI can be used as a means to *combat venereal diseases* in cattle such as trichomoniasis and campylobacteriosis (see Book 1). In fact an important reason why AI in cattle was introduced in most European countries was to combat venereal diseases.
- Application of AI *extends the use of good sires*.
  A bull used for natural service breeds considerably fewer females than a bull used for AI. In natural service one ejaculate is used to breed only one female whereas in AI each ejaculate can be diluted, stored, and used to breed many females. With AI one bull can breed from 20 000 up to 100 000 or even more cows per year depending on semen quality, processing of the semen and on insemination techniques.
  Thus artificial insemination provides the means by which a sufficient number of offspring can be bred from a bull in a relatively short time in order to record the performance of this bull.
  Once the bull has been genetically proven, then AI alone can assure the fullest use of this bull.
  Hence AI is a method by which bulls can be genetically tested and by which the most prominent proven bulls can be used intensively for the production of the next generation. The cattle population can thus be rapidly improved provided that sound selection methods are applied. This is in fact by far the most important reason for the widespread application of artificial insemination in cattle.
- Artificial insemination permits the *use of different bulls* and even bulls of different breeds within one herd.
  This enables herd owners to breed individual cows to selected sires according to the breeding goal and the genetic make-up of dams and sires.
- In dairy herds where income derived from milk production largely exceeds income from beef production, the application of *AI can be cheaper* than keeping a bull oneself.
  The costs of feeding and of depreciation of even inferior bulls can be higher than the fees for the insemination service, apart from the fact that AI provides the farmer the use of genetically better bulls.
- In the (sub)tropics especially *dairy smallholders* can take advantage of AI since it permits them to keep dairy crossbred animals and to apply criss-crossing with, for instance, Sahiwal and Friesians.

## 1.2 Limitations and negative aspects of AI

- Even under the most favourable conditions the *conception rates of AI are considerably lower* than those of natural service from bulls allowed to run free with the herd, and slightly lower than those of natural service by ‘hand mating’.
  Under less favourable conditions the conception rates of AI easily become so low as to cause serious economic losses to herd owners.
  Conception rates of AI largely depend on the fertility of the bulls in use, the semen collection method which is applied and on insemination techniques. Acceptable conception rates of AI can only be achieved by continuous monitoring of results and by continuous supervision of methods and techniques by skilled and knowledgeable persons.
- The success of an AI service also depends very much on *the management of the herds* which are served.
  The owners of these herds or their personnel must *be able to detect heats accurately* and in fact do so, as nobody else can do this for them.

Artificial insemination is therefore **not** applicable where cattle cannot be kept under close supervision or where basic knowledge amongst farmers about heat detection does not exist.

- Experience in many countries has shown the *serious risks of AI* with semen from bulls which are not *thoroughly tested for the absence of undesirable genes* (traits).

  The widespread use of bulls which are not properly tested under local environmental conditions (or conditions similar to those prevailing locally), can easily lead to the introduction of undesirable genes in the population.

  Therefore the application of AI is only warranted in situations where the bulls used for AI are properly selected under **local** environmental conditions.

  This is very important in cases where AI is used to cross an existing local cattle population with bulls of exotic dairy breeds in order to increase the milk production ability of the cows.

  Outcrossing an existing cattle population with foreign breeds not only leads to the introduction of the genes from the foreign breed; it also causes a loss of valuable adaptive genes in the local population.Genes which enabled the local cows to survive and to reproduce under local conditions.

  Foreign breeds, by natural and human selection, have been adapted to the environmental conditions prevailing in their country of origin. But they do not necessarily possess the genetic make-up which allows them to thrive under completely different conditions of climate, feeding and management.

  Although under optimum management conditions European dairy breeds like Friesians and Jerseys do reasonably well under a variety of climatic conditions, the unlimited use of foreign dairy breeds in tropical areas will almost certainly cause an introduction of undesirable genes and a loss of desirable genes. This could, for instance, result in cows which are more susceptible to heat stress and tropical diseases.

  Unless the bulls used for widespread use of AI are carefully selected under local environmental conditions, the resulting progeny may in fact become less productive on a lifetime basis than the indigenous cows.

- Proper application of AI will lead to improved animals capable of producing more milk than the original cows. However, such animals are usually more susceptible to stress and *they require improved feeding and management methods*. Therefore the introduction of AI will only lead to the expected results in situations where animal health care, feeding and management do not constitute limiting factors in the drive towards increased productivity.

  So, parallel efforts are required to strengthen the existing animal health service and to improve feeding and management of the new stock.

- *Skilled inseminators are required* for optimum conception rates.

  A minimum number of at least 1000 inseminations per year is necessary for inseminators to retain their skills. This restricts the application of cattle AI to areas with a sufficiently dense cattle population or to very large isolated herds.

  A network of roads throughout the area of operation is also essential, in order to make full use of the inseminators' time and to permit the distribution of semen supplies to the various insemination (sub)centres.

- Unless appropriate measures are taken, AI can be a means of *spreading infectious diseases.*

  The bulls in the AI stud should be free from infectious diseases.

  All AI activities should be under continuous veterinary supervision to prevent diseases from being spread through artificial insemination.

## 1.3 What is necessary for a successful AI programme?

The basic goal of any AI programme should be *genetic improvement.*

Improved cattle, however, require high standards of nutrition, health care and management for survival, production and reproduction. In many cases this requires the development of plans for increasing and improving the production of roughage and concentrates needed to realize the increased production capacity.

In areas with small (dairy) farms and traditional cattle management, the establishment of an *appropriate dairy extension service* is required to supply knowledge to the farmers about improved dairy management (housing, nutrition, fodder production and conservation, handling and milking, disease prevention, breeding, a.o.). Without improved dairy management genetic improvement makes very little sense.
**The value of textbooks like the Modern Dairy Farming series, and the underlying text about reproduction in dairy cattle, is limited. However, lots of errors can be avoided by reading through these texts; at (almost) no cost (!) compared to the amount of money involved in the importation of just one exotic dairy heifer or the costs of sending just one person abroad to attend a dairy farming training course.**

Also an appropriate *animal health service network* is required because genetically improved cows tend to show an increased susceptibility to diseases.
Major infectious diseases should be controlled or eradicated before the introduction of exotic susceptible breeds into the area.

*Careful planning of a breeding programme* is another prerequisite before the start of an AI programme.
Before any genetic improvement programme is embarked upon it is essential that its planners establish the ultimate goal of the programme. This may differ greatly from region to region, depending on local environment, the role played by cattle in the economy and ecology and limiting factors such as labour, land or feed.

An important limiting factor in determining objectives for breeding plans is *the climate.* Improved dairy breeds thrive in moderate climates but subtropical and tropical areas certainly have special problems when it comes to cattle breeding, maintenance and health.

*European dairy breeds can survive, reproduce and produce in hot, dry areas,* provided that they are protected against environmental hazards by the provision of shade, regular spraying or dipping, a supply of cool drinking water, being fed optimally adapted diets, reliable heat detection, overall good hygiene and management and an intensified veterinary care.

In *permanently hot and humid areas,* however, pure breeds of European cattle do not thrive. They may not even survive.
Especially affected is the fertility of the cows and the viability of the calves.
Under such conditions experience has shown that *crossbreeding* with local cattle to meet local conditions is normally the most practical course of action. Generally the resulting crossbreeds are superior to both purebred parent populations.

In such a programme AI bulls are bred as crossbreeds *in nucleus herds* by crossing carefully selected dams of indigenous breeds with semen from selected bulls of exotic breeds. The resulting male offspring is used to perform inseminations in the field.
Gradually, after thorough experience with the resulting offspring under field conditions, the proportion of exotic genes can be increased up to 3/4 or even 7/8 but upgrading should proceed with caution up to the optimum genetic level under the prevailing conditions.

However, it is extremely difficult to accomplish a controlled crossbreeding programme with AI.
In many cases the introduction of exotic breeds through AI has resulted in an uncontrolled and unlimited outbreeding of the indigenous population and consequently in disappointing results in the long run.

Finally, an increase in milk production requires an *appropriate milk marketing system* including milk collection, processing and distribution facilities.

Without possibility to sell the increased milk production at reasonable prices, farmers will have no incentive to increase the milk production of their cows and thus AI will fail to acquire broad acceptance amongst farmers.

Many AI programmes throughout the world were started without planned breeding programmes or without the necessary infrastructure having been set up first and consequently did not result in a considerable increase in production.
Such programmes invariably lead to a waste of money and effort because AI programmes, whether successful or not, are always expensive and labour-intensive.

AI can only give a considerable return on investments in situations where the prerequisites as summed up in this chapter have been taken into account.

# 2 Incorporation of artificial insemination in genetic improvement programmes

### Selection of bulls

The introduction of AI requires careful planning of breeding goals adapted to local circumstances and careful establishment of a breeding programme to achieve these goals.
Therefore any AI organisation should be integrated in a genetic improvement programme.
Cattle geneticians should decide on the choice and selection of the bulls.

The selection of the bulls to be used for AI should always be based on their breeding values in relation to the overall breeding goal of the AI programme.

Basic principles of the assessment of breeding values of individual bulls can be found in PRINCIPLES OF DAIRY SCIENCE, by G.H.Schmidt, L.D.Van Vleck and M.F.Hutjens (Prentice-Hall Inc., New Jersey, USA).

### Importation of semen

Quite often AI in many developing countries started with the use of imported semen.
Frozen semen from high quality dairy bulls of 'Western' countries was and still is donated to developing countries under the FAO Bull Semen Donation Scheme (IBSDS). This programme, introduced as long ago as in 1971, initially aroused great interest and several semen-donation projects have been implemented since then.
However, the overall results of these projects were discouraging. In semen importation it is assumed that the best bull in the exporting country will also be the best bull under the local conditions in the importing country. Quite often this is **not** the case, especially when semen is imported from countries where bulls are selected under quite different climatic and environmental conditions.

Large-scale importation of semen is only a realistic approach when preliminary trials have shown that the exotic gene pool does well under the local environmental conditions and management. Furthermore, the breeding goals of both the exporting and importing country must be similar. And sufficient foreign currency must be available to guarantee a regular and uninterrupted importation of semen.

### Nucleus herds

A far better approach is to establish 'nucleus herds' locally and to breed AI bulls from these herds.
In these herds the animals can be selected for their adaptation to the local environment even though their number might be too small to permit the immediate start of bull progeny testing programmes.
Whether the females in these herds should be carefully selected outstanding cows of indigenous breeds, **or** imported cattle from exotic breeds, should depend largely on the quality of tbe existing indigenous breeds, the breeding goals and the prevailing climate, and on ecological and economical conditions.

Especially in hot, humid climates and in areas with primitive dairy management, bulls bred by crossbreeding Bos indicus with Bos taurus breeds will often be better suited for widespread use through AI than purebred European dairy bulls.

By the production of crossbred AI bulls, the unlimited and uncontrolled spread of exotic genes throughout the cattle population can be prevented. The same applies to the undesirable disappearance of valuable adaptive genes in the indigenous stock.
However, it requires careful breeding plans in the nucleus herds, geared to preserve a purebred stock of selected indigenous cattle.

Nucleus herds for the production of AI bulls should be sufficiently large to permit a reliable selection of bull dams in the herd.

In the nucleus herds the animals should be kept under optimum conditions of feeding, housing, care and management so that the cows can produce as near as possible up to their genetic capacity. Under such circumstances the performance and the production of the individual animals in the herd will reflect their individual genetic merit. Thus the individual animals can be ranked on the basis of production, conformation and disposition, making a selection of individual animals within a herd possible.

Selection of individual outstanding animals within the nucleus herd under optimum management conditions is only possible when there is *a reliable registration of each individual animal in the herd.* The registration of each animal should include pedigree, growth and performance, fertility and diseases, dairy disposition and character, conformation and, most important, an *individual production record* of milk and butterfat produced by the animal. Only in this way can a reliable selection based on production, conformation and dairy disposition (ie. ease of milking, character) of individual animals be carried out.

The most outstanding animals in the nucleus herd are used as bull dams.
For every bull needed in AI, three or four bull dams have to be selected as one cannot expect each bull dam to produce a live and sexually normal bull calf.
The bull dams should be inseminated with semen from the most outstanding progeny tested AI bulls available.
The male offspring will then have the highest possible genetic capacity to produce offspring with a high level capacity for production and performance.
They can be selected on growth rate and conformation in a *bull calf rearing unit* up to sexual maturity and the best ones can be taken into use as AI bulls for the upgrading of the local cattle population.
In nucleus herds destined for the production of bull calves in programmes for outcrossing or breed replacement, the female offspring of the bull dam is kept in the herds for replacements.
However, in nucleus herds destined for the production of bull calves for upgrading purposes, the female offspring of bull dams has either to be sold to other farms (replacement of the female stock in the herds by acquisition of purebred stock from elsewhere) **or** they should be inseminated with semen from crossbred bulls in order to maintain the optimum degree of upgrading.

### Progeny testing schemes
Progeny testing of AI requires well organised systems for pedigree registration and individual milk recording.
The use of rather sophisticated electronic data processing equipment for the calculation of breeding values is recommended, although judging based on average yields of daughter groups can provide sufficient information.

Systems for progeny testing AI bulls are described in PRINCIPLES OF DAIRY SCIENCE mentioned before.

If such an approach is decided upon, the first step is to institute *a milk recording system* and a *breeding results recording system.*
These systems should include a large proportion of the cattle population in the area covered by AI as the size of the registered and production recorded population is a controlling factor.
For example, to test ten bulls a year requires about 5,000 cows in registered and controlled herds. Assuming that half of the cows are mated to proven sires, the total population of recorded and inseminated cattle should be at least 10,000.

In areas where a sufficiently large cattle population is registered and milkrecorded, planned mating systems for the production of future AI bulls can be introduced to replace the system of nucleus herds.
By intra-herd comparison based on genetic principles, the most outstanding dams from the entire milk-recorded population can be selected as bull dams and mated to the most outstanding progeny-tested sires to breed the future generation of AI bulls.
So, the introduction of a milk recording system not only permits the institution of a progeny testing system for AI bulls, but also an improved system for the acquisition of future AI bulls by selection of bull dams in a larger population.

However, in areas with a *very low average herd size,* or with primitive dairy herd management, the introduction of a milk recording system is not a good proposition:

- usually there is a lack of interest on the side of the farmers (and what incentives can be offered?);
- in the majority of smallholders' herds poor management, low feeding levels, sucking of calves, shortened lactation periods and other things will often cause a too low heritability of milk production as measured by milk recording; so, milk recording under these conditions has no value at all for genetic selection.

Therefore, for genetic improvement one should here rely on careful selection of AI bulls *based on their pedigrees.*

# 3 Organisation of an artificial insemination service

## 3.1 General considerations

Acceptance of AI by herd owners and good conception rates both depend largely on a *regular, daily* availability of the AI services. Farmers demand an AI service that operates every day of the year.
There are many ways to organise an AI field service so that farmers' demands will be met. The important principle is that the organisation must be carefully adapted to local conditions and that sufficient reserves are built in to guarantee a *reliable service* and *acceptable conception rates.*

For the continuity of the services it is of vital importance that all necessary requirements, such as finances, materials, facilities and skilled personnel are continuously available. An uninterrupted, optimum field service must be guaranteed.

In the past too many AI programmes have failed simply because of inadequate planning.
Failures of first attempts discourage everybody concerned from trying again even when conditions are made much more favourable.

#### Choice of area to be covered

Areas with the best potential for production improvements must be given priority for AI development.
Facilities for disease control and for collection and processing of milk must be available in principle, in areas selected for AI establishment.

The basic rule is to maintain adequate financial and personnel reserves and not to stretch resources too far, even when pressure is applied to do so. Maintaining *continuity of operation* should be the primary consideration, even at the expense of complete coverage of the area selected.

A successful AI operation means enthusiastic cooperation on the part of the farmers. An area in which a majority of farmers is eager to improve their herds by application of AI is to be preferred over a larger area in which only a minority of farmers is willing to participate.
Experience has shown that seducing or even forcing farmers to apply improved breeding methods always leads to mistrust of the farmers involved and thus to negative results.

In areas where bulls are allowed to run free with the herds or have free access to the females, AI is **not** applicable because of lack of knowledge amongst farmers on how to detect heat in cattle.
Also in areas with very primitive farm management systems or in areas where farmers stick to tradition (reluctance to accept new ideas), the introduction of AI will most likely prove to be very disappointing. In such areas an overall change in farmers'attitudes and management practices is required before AI can be successfully introduced.

#### Combining AI with other services

Many attempts have been made in the past to combine AI with other services, in situations where inseminators were not fully occupied with their proper tasks.

An obvious combination is AI and animal health. However, if the inseminator must also participate in ordinary veterinary services, these services can take precedence and this may result in a slow-growing or stagnating AI service.
Moreover, this combination easily leads to confusion amongst farmers. They may consider AI as a gynaecological rather than as a genetic measure.
AI is by no means a gynaecological treatment as conception rates of AI are generally lower than those of natural matings.

Optimum conception rates of AI require that only gynaecologically healthy cows are presented for insemination, and not those that have failed to conceive after natural mating. Therefore it is important that a clear distinction is made between gynaecology and AI.
Veterinarians and animal health workers should not inseminate cows; inseminators should not perform gynaecological treatments. When inseminators detect abnormalities, they should advise farmers to consult a veterinarian for proper treatment prior to insemination.

Combining AI work with general animal husbandry advisory work is another possibility. However, this combination can also lead to delays and interruptions in regular daily AI services.

The recommended procedure is to use *full-time, specialised* inseminators. This makes it possible to perform a maximum number of inseminations per day. It also helps the inseminator to maintain his manual skills.

## 3.2 Organisation of field services

### Locally stationed inseminators

The inseminators are stationed individually and the farmers bring their cows to the insemination point.
At first sight this seems to be an easy and cheap arrangement but there are many obvious disadvantages:

- it requires a large number of inseminators and insemination centres as one cannot expect farmers to walk long distances with their cows in heat;
- the number of inseminations per day may be very low and therefore inseminators may loose their skill (one thousand inseminations per year are considered a minimum);
- a dense network of individually stationed inseminators is difficult to supervise and difficult and expensive to supply with semen and requisites;
- losses of semen (when fresh semen is used) or of liquid nitrogen may be high.

In general, individually stationed inseminators *are only recommended in situations* where they are employed directly by a local group of farmers in an area with a dense cattle population or where they are working with one or more large-scale farms.

### Travelling on request

The inseminator goes to a farm whenever a cow is found to be in heat.
Here travelling tends to be uneconomic since it cannot be planned ahead of time.
Moreover, a single insemination may take several hours and because of this other farmers may have to wait.
Conception rates are generally low (inseminators do not get enough practice).
Experience with this system has generally been discouraging.

### Roadside crush system

An obvious variation on ‘travelling on request’ is *to have the inseminator move along a scheduled circuit within his area every day.*
Farmers are informed of the period of time the inseminator will remain at each ‘insemination point’ which may be a roadside crush.
Roadside crushes are simple wooden constructions with two lattice frames between which the cow is kept in a fixed position during insemination.
The crushes can be constructed by the farmers themselves, and located a few kilometres apart.
The inseminator travels daily along the road, following a predetermined schedule and visiting each crush at a predetermined time.
This roadside crush system can even be used in difficult or densely populated cattle areas, where the inseminators have to travel by bicycle or even on foot.

Naturally the *means of transport* must be carefully adapted to local weather and road conditions. Motor transport is much more expensive than travelling by bicycle, by ox cart or on foot. However, coverage and efficiency are better. The possibility of breakdowns must be weighed carefully against the improved efficiency of motor transport.

Ordinary motor cars may not be able to pass in the rainy season and here four-wheel drive vehicles are required to ensure a regular daily service.

A rough indication (that is all!) of output per day:

*Table 1:*

| | **coverage** | **number of crushes** | **number of inseminations** |
|---|---|---|---|
| car | up to 150 km | 30 - 70 | 10-25 per carround (up to over 60 has been proved possible) |
| motorcycle | 80 km | 10-40 | |
| bicycle | 40 km | 5-20 | |

To compensate for breakdowns, maintenance and minor repairs, about 1.3 car or 1.5 motorcycles or 1.1 bicycles should be available per daily round.

**Farm-visit programme**

A farm-visit programme is an expansion of the roadside crush system. This programme is mainly suited to large-scale dairy farming areas with good roads. Farmers can contact inseminators by telephone, if available, or by flags or signs displayed along the road.
Where telephone connections are available, the planning of the inseminator's rounds can be flexible; where the flag system is used, fixed daily rounds have to be planned as in the roadside crush system.

As to the **choice of system,** both the roadside crush system and the farm-visit system are more appropriate and efficient than any other system. They ensure maximum employment of inseminators and reduce investments in materials and equipment. They avoid wastage of semen where fresh semen is used and also avoid wastage of liquid nitrogen where frozen semen is applied.

Moreover, these systems allow the establishment of *subcentres,* from which up to fifteen inseminators operate covering a large area.
This greatly facilitates supervision and the supply with semen and liquid nitrogen.

## 3.3 Main centre and subcentres

The most successful organisational structure for an AI operation is the establishment of a main centre with several subcentres situated at strategic positions throughout the area of operation.

The *main centre* serves as the central semen distribution centre.
It supervises the activities of the subcentres, assesses the quality of the semen issued and acts as the administrative centre.
In most cases it also serves as a bull semen production centre.

The *subcentres are regularly visited by AI supervisors* who report to the main centre. They should visit each subcentre for a few days each month. On these visits the supervisors should spend much of their time on examining AI records and considering financial aspects including transport costs and liquid nitrogen use.
They should also follow up individual inseminator performance in the field, by checking on time schedules, care and correctness of AI records, insemination techniques, handling of AI equipment, use of vehicles and even the attitude of inseminators towards farmers.

Moreover, they should check on stores, use of transport facilities, supplies of semen, recording of AI fees, transport management, the balancing of the number of semen doses in relation to inseminations performed, and so on.

AI supervisors should be recruited from those persons who have established a particularly good field performance record.

Close cooperation between supervisors and inseminators is extremely important.
The attitude of supervisors should basically be that of advisors, staff trainers and promoters of AI service, rather than of superior controlling officers.

**Subcentres**
The actual inseminations are performed through the subcentres.

It is advantageous to keep the number of subcentres as low as possible. Each subcentre should perform a sufficient number of inseminations to keep the inseminators fully occupied.

Each subcentre is managed by a *field officer.*
Field officers hold the key positions in any AI field service. They are responsible for the daily activities in the subcentre's area and report to the AI supervisor.

Field officers should undergo basic AI training of the type given to inseminators, plus at least one six-weeks course in organisation and management of AI schemes.
It is advisable to set up regular in-service seminars and meetings for field officers.

Generally, field officers should be recruited from those inseminators showing high conception rates and exceptional dedication to their job.

The number of inseminators required per subcentre should be about 60% above the number of daily rounds. This is based on a 240-days working year, with reserve for in-service training, sick leave and other contingencies.
One field officer and one clerk will usually be needed in addition.

Experience has shown that granting financial incentives based on quantity and quality of the inseminations performed can have beneficial effects on the expansion of the AI service and on conception rates obtained by the inseminators. Incentives could, for instance, be based on the number of calves produced per inseminator.
The housing of field staff should be considered carefully. The local centre is often located in a rural area where housing may be hard to find. Staff motivation is vitally important, however.

## 3.4 Financing AI services

In most Western countries AI organisations are self-supporting and financially independent. They operate without government subsidies as farmers' cooperatives or as private enterprises.
The running costs are met by charging the users of the service realistic insemination fees.

Provided proper breeding plans are applied, the real costs of any AI service are always lower than the benefits from increased productivity through the use of selected high-quality bulls.

Most AI organisations in Western countries charge dairy cattle owners *an insemination fee for each first insemination.*
Followed, if necessary, by a restricted or even unlimited number of **free** repeat inseminations (in case the cow fails to conceive).

Repeat inseminations at more than six months after a preceding insemination are generally considered as being first inseminations.
Often, within one AI organisation, different insemination fees are applied, depending on the quality of the bull used. Or a distinction is made between the price of the semen used and the actual insemination costs.

In many countries elsewhere, however, the situation is different.
For one reason or another AI organisations are usually government services or organisations heavily subsidised from public funds.
The farmers are usually insufficiently organised or have insufficient capital to set up their own AI organisation.

Yet it is strongly recommended to charge the users of any AI service *at least a small insemination fee.* Demanding of even a small insemination fee will prevent farmers from presenting cows that are not likely to conceive and this will help to obtain good conception rates.

The simplest and cheapest way to collect insemination fees is to have them collected by the inseminators ie. to ask the farmer to pay the inseminator in cash for every first insemination.
Collecting the insemination fees by sending the farmers invoices requires a more complicated accounting system and is more expensive.

The *ultimate goal of any AI organisation should be self-sufficiency,* which it can best realize by charging farmers realistic insemination fees, as only this approach will force the AI service to provide the best possible service at the lowest possible cost.
There is another important reason for charging the farmers an insemination fee per first insemination: it is an indispensable measure to obtain reliable conception rates. Only where farmers have to pay for a first service, with repeat services free of charge, one can expect that the majority of animals that failed to conceive will be presented for a (free) repeat service.

Where 'bribing' of inseminators occurs, this usually indicates that insemination fees are too low and that the service is poorly organised.
Much can be done by raising the insemination fees, by improving the service and by paying the inseminators higher wages, preferably performance-related.

# 4 The semen production unit

Where fresh semen is used, an AI organisation needs at least one bull per thousand cows to be inseminated; this allows for temporary cessation of the semen production of individual bulls.

With frozen semen fewer bulls are needed, but at least one bull per three thousand cows is recommended.

When there is no bull progeny registration and testing system, it is strongly recommended to replace the AI bulls frequently in order to avoid the risk of inbreeding and to restrict the spread of possible undesirable genes throughout the local cattle population (this may not be noticeable). For the same reasons the bulls in the AI centre should not be interrelated.

Even in the smallest AI operation there should be four bulls at least; with a smaller number one risks not to have bull semen available continuously.

### Housing

Bull pens should measure at least 4 × 4 m and give free access to an exercise lot in the open air of approximately 4 × 8 m.
Both the floor of the pen and the exercise lot should be made of concrete to enable regular cleaning.
In one of the corners of the pen the construction of a resting place with a surface of at least 1.20 × 2.00 m is recommended.
This resting place with clean straw bedding will keep the bull clean.

However, there is ample evidence that bulls remain in good health and maintain a satisfactory level of semen production and fertility when allowed only the freedom of their pens provided that these pens are sufficiently large.

Tying of the bulls in *stalls* can also be satisfactory provided that sufficient attention is paid to size and construction of the stalls.
Keeping the bulls tied in stalls saves considerably in building and labour costs. But it requires regular hoof care (trimming). And the stalls should always have a thick, clean straw bedding to prevent damage to skin and joints.
Stall housing is better than housing the bulls in loose pens which are too small.

Special attention should be paid to *ventilation.* Bull houses must be kept as cool as possible.
When constructing bull houses one should realize that both libido and semen production capacity of bulls are adversely affected by high temperatures.
Even in temperate climates some AI centres have airconditioned bull houses (temperature below 15 °C) for optimum housing of their valuable bulls.

### Feeding AI bulls

Young bull calves should be fed a liberal, well-balanced ration in order to obtain maximum growth.
Excess feeding of high-fibre rations should be avoided as this retards growth and causes paunchiness.
Cool, clean water should always be available to the calves. Supply of water by automatic drinking bowls is recommended.

Properly fed bull calves should reach sexual maturity at 12 to 15 months of age.

After the bull has reached the age of 16 to 20 months feeding should become less liberal. At this age the growth rate decreases considerably; the only aim of feeding should now be to keep the bull in good breeding condition.

A breeding sire should never become fat because this has a negative effect on semen production and fertility.

Excess roughage in the ration should be avoided because it leads to paunchiness which adversely affects the sex drive. The ration should be well-balanced and adjusted to the weight of the bull.
Minerals and vitamins in the ration should be properly balanced. Special attention must be paid to carotene in the rations; lack of carotene has proved to affect fertility adversely.
Young, green feedstuffs normally contain enough carotene (vitamin A).

Sudden changes in the composition of the ration should be avoided as they can affect the quality of the semen adversely.
Semen quality changes caused by sudden fodder changes are often not immediately noticed because their effects become apparent after 4-6 weeks only, due to the fact that ripening and maturation of male cells inside the male genital tract takes several weeks.

AI bulls should continuously have free access to cool and clean drinking water.
In hot climates mature bulls can drink up to 70 litres of water per day. Installation of automatic drinking bowls is recommended.

### Care and handling of the bulls

Bulls should be kept clipped and groomed and their pens should be kept clean and well-bedded.
The long hairs at the sheath should be clipped regularly and especially the underside of the belly should be kept clean in view of hygienic semen collection procedures.
However, washing the bulls immediately before semen collection is not recommended; for hygienic reasons bulls should be dry during semen collection.

Special attention should be paid to *regular hoof trimming.*
Negligence affects the sex drive and thus decreases semen production.

For safety reasons AI *bulls should be provided with nose rings.* Ringing is best done at an age of 6-8 months.
Many AI centres also dehorn the bulls in the stud for safety reasons.
As bulls are always dangerous in principle they should be handled with care. At least two persons (bull attendants) should always be present when bulls have to be moved or handled, so that one can assist the other in dangerous situations.

### Health tests and disease control

The secondary aim of artificial insemination is disease control.
So, AI centres must ensure that bulls are free from infectious diseases prior to their introduction in the centre.

Particular attention must be paid to those diseases that can be transmitted through semen, such as tuberculosis, brucellosis, IBR/IPV, leucosis, leptospirosis, campylobacteriosis and trichomonasis.

Before bulls enter the stud, they should be checked for the presence of the above diseases. Only bulls that are free from these diseases should be accepted.
Moreover, regular health checks (at least twice yearly) are necessary to ensure the maintenance of the disease-free status of the bulls of the centre. Any bull stud should be under continuous veterinary supervision.

Without sanitary precautions AI can rapidly spread contagious venereal diseases rather than being a means for *combating* such diseases.

# 5 Semen collection and processing

Semen collection methods are described in FERTILITY AND INFERTILITY IN DOMESTIC ANIMALS edited by J.A.Laing, Baillière Tindall - London, and in PRINCIPLES OF DAIRY SCIENCE mentioned in Chapter 2.

Semen collection should take place in a special area. This ‘arena’ should be large enough to permit safe handling of several bulls at a time.
It should have a roof (protection from rain and sunshine) and it should be in the direct vicinity of the semen processing room to avoid unnecessary delay between semen collection and semen processing.
The floor of the semen collection area should be paved. Semen collection on a dusty or muddy floor will invariably result in contamination of the collected semen.
The floor should not be slippery.

Other bulls or bullocks can be quite satisfactorily used in semen collection (as teaser animals).

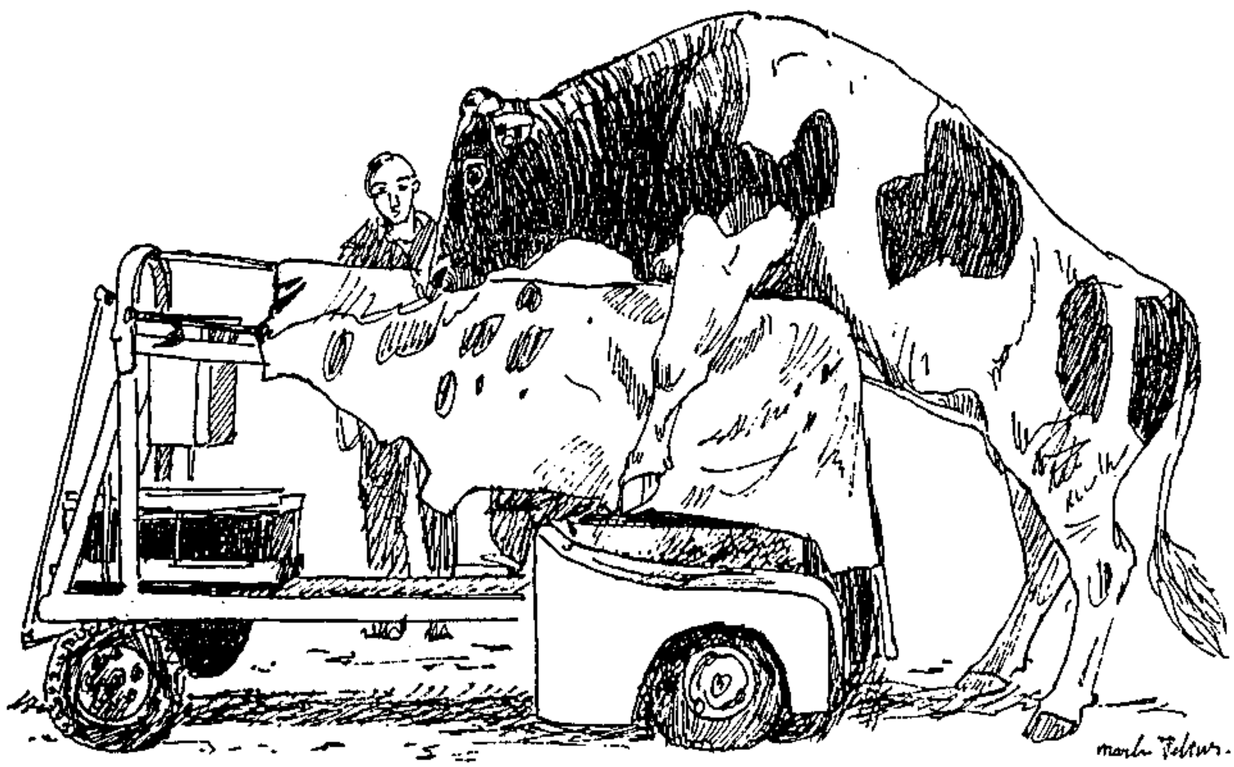

*Figure 1: Bull mounts riding dummy cow*

The use of dummy cows with a built-in seat for semen collection has the advantage that bending of the penis is no longer necessary. This intensifies thrust and ejaculation which has a favourable effect on the quantity and quality of the semen which is collected.

*Artificial vaginas* can be recommended for general use in AI centres.

This cannot be said of electro-ejaculation because it is rather dangerous for bulls as well as for bull attendants as there may be muscular spasms.
Moreover, the quality of the collected semen is generally very poor due to the presence of urine and smegma (ie. secretion in folds of the prepuce) and of an increased amount of fluid from the accessory glands.
And experience has shown that electro-ejaculation shortens the productive lifespan of bulls.

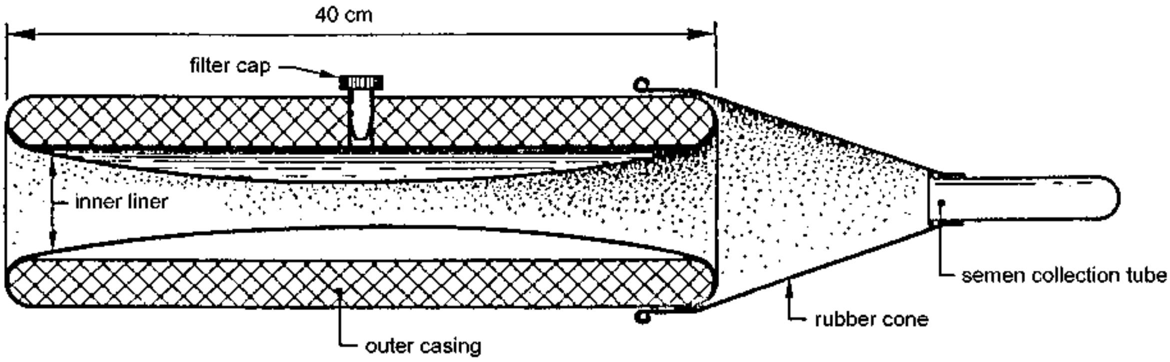


*Figure 2: Artificial vagina for the collection of semen of AI bulls*

Artificial vaginas should be *properly sterilised* in order to prevent any possible spread of infections between bulls within a centre and to avoid contamination of the semen.
This means that after each use they should be thoroughly cleaned and sterilised in boiling water. Then they should be stored in a clean, dust-free place.
The use of a specially temperature-controlled cabinet for the storage of artificial vaginas can be recommended.

**Collection frequency and pre-collection stimulation**
It is generally agreed that, when proper semen collection techniques are applied, the average sexually mature bull can be expected to ejaculate at least four times a week without appreciable negative effects.
More ejaculations do considerably increase the amount of semen produced by the bull but require very good collection and stimulation procedures because the bulls's sex drive tends to decrease with this.

Pre-collection stimulation is recommended; this increases both the quality and the quantity of the ejaculated semen.
A commonly applied practice is to allow the bull to perform two false mounts, followed by 1-2 minutes of active restraint, and again one false mount, before semen is finally collected. However, since bulls show great variations in behaviour and temperament, one cannot expect one rigid system to be equally satisfactory for all bulls.
The main aim should be to accustom the bull to a set routine for semen collection. At collection time the important thing is to stimulate the bull to mount the teaser and to ejaculate, in the manner which has been found to initiate the copulatory reflex of the bull in question.

What should be avoided in all cases:
- rough treatment
- sudden moves or loud talking
- artificial vaginas which are either too cold or too hot; their temperature should be 40 to 45 °C

Incorrect temperatures of the vagina will in the long run make the bull refuse to mount and to ejaculate.

**Semen evaluation**
Semen evaluation methods are described in FERTILITY AND INFERTILITY IN DOMESTIC ANIMALS mentioned above.
There would seem to be a significant correlation between fertility and the following semen characteristics:
- semen morphology (the form of the male cells)

- heat-resistance of semen
- percentage of male cells with intact acrosomes (tips)
- number of motile male cells which are inseminated (ie. cells capable of motion)

Before semen is diluted and processed the following characteristics should be examined at least:

- volume → ejaculates with a volume of less than 1 ml should be rejected (not be kept)
- colour → ejaculates with abnormal colour should be rejected
- consistency → ejaculates with abnormal consistency should be rejected
- concentration → ejaculates with less than 500 million male cells per ml should be rejected
- mass motility → ejaculates without any wave motion should be rejected
- individual → ejaculates with less than 60% motile cells should be rejected motility

Semen morphology:

- where semen morphology is assessed with a phase-contrast microscope, a gross examination of the morphology can be performed during the assessment of individual motility; samples showing abnormal morphology should be morphologically examined;
- where no phase-contrast microscope is available, all semen samples should be morphologically examined prior to processing; ejaculations with more than 20% abnormal cells should be rejected.

After processing and before it is issued for insemination, semen should at least be tested on the following:

- *survival rate* semen samples in which less than 40% of the cells survive after processing and storage, should be rejected;
- *number of living cells* samples with less than 6 million motile cells per insemination doses should be rejected;
  the minimum number of motile cells per insemination depends largely on the insemination techniques which are applied; so, in AI organisations with insufficiently trained inseminators the minimum number of living cells should be much higher than 6 million;
- *heat resistance* semen samples in which less than 25% of the initially living cells survive after four hours storage in a waterbath at 37 °C should be rejected.

**Semen processing**

Semen processing for the production of liquid semen and handling, storage and distribution of liquid semen are extensively described in FERTILITY AND INFERTILITY OF DOMESTIC ANIMALS mentioned above.
Basic principles for the production of frozen bull semen can be found in the same book.

In recent years the techniques for freezing semen in plastic straws have replaced almost completely all other semen techniques.
Standardised processing techniques for the production of bull semen in straws have been worked out.
On request, the manufacturers or suppliers of equipment for freezing in straws can organise practical, on-the-spot demonstrations to familiarise the laboratory workers with the techniques involved.
It is strongly recommended that in the production of semen frozen in straws, the prescriptions of the straw and equipment suppliers are meticulously followed.

The production of frozen semen requires *much higher investments* in laboratory equipment than the production of liquid semen.
Moreover, machinery and instruments necessary for the production of frozen semen can break down.
*Skilled craftsmen* for the repair of the rather complicated machinery and equipment must be readily available before the decision is made to switch from liquid semen to frozen semen production programmes. There must also be an ample stock of spare parts.
Refer to following chapter for more details.

# 6 Fresh semen or frozen semen

The use of frozen semen has the following *advantages:*

- It can be stored for long (indefinite) periods without losing its fertilising capacity and it can be transported over unlimited distances.
  In contrast, fresh semen, even under optimum conditions of buffering and storage, deteriorates rather rapidly and can be used for a maximum period of four days only.
  Thus, with frozen semen wastage is avoided and consequently bulls can be used more efficiently.
- Frozen semen can be distributed to subcentres at intervals of several weeks or even months rather than the three-day-period required by fresh semen; this reduces transportation costs considerably.
- The use of frozen semen also reduces the number of bull semen production units required to cover a given area because frozen semen can be transported in bulk over unlimited distances.
- The use of frozen semen provides increased versatility in breeding plans because a variety of bulls can be made available.

But there are *negative aspects* as well:

- High investments and high running costs are involved in keeping the product (the semen) *continuously* at a very low temperature.
  Keep in mind that an exposure for just a few seconds to temperatures higher than minus 120 °C can already damage frozen semen!
- The constant need for cooling of frozen semen requires a *reliable* liquid nitrogen ($LN_2$) supply, in sufficient quantities.
  A breakdown in the nitrogen supply can have disastrous effects in AI organisations working with frozen semen.
  Careful planning of liquid nitrogen acquisition and distribution along with ample reserves to overcome temporary breakdowns are essential in a frozen semen operation.
- Frozen semen requires *more skill and accuracy* both from laboratory workers and from inseminators.
  It is easier to obtain acceptable fertility results with fresh semen than with frozen semen.
  Laboratory workers as well as inseminators must therefore be re-trained when an AI programme switches from fresh semen to frozen semen.
  Special attention must be paid to inseminators' techniques because the successful use of frozen semen depends largely on correct thawing and insemination procedures.
  However, experience has shown that it is possible to obtain fertility rates which are quite comparable with those obtained from fresh semen provided that inseminators and laboratory personnel are properly (re-)trained and informed.

### Acquisition and distribution of liquid nitrogen

In technically advanced countries and in areas around industrial centres in less advanced countries, liquid nitrogen can often be acquired through bulk suppliers or directly from gas producing plants.
Where this way of liquid nitrogen acquisition is adopted, a *continuous* operation of the gas producing and delivering plant must be guaranteed and road or rail transport must always be available.
However, sufficient quantities of liquid nitrogen should still be kept in store at the semen production unit as a reserve in order to overcome possible breakdowns in the delivery system.

Where outside supplies are not absolutely guaranteed or not available at all, on-site production of liquid nitrogen is recommended.
Compact, self-contained liquid nitrogen producing plants are available on the market, with production capacities ranging from about 1000 to 3500 litres per week.
These plants can be installed at any site where reliable electrical power is available.
But also in this case precautions should be taken to ensure a *continuous availability* of liquid nitrogen:

- the plant must be installed and operated by trained and knowledgeable personnel, strictly according to the instructions of the manufacturer;
- it must be serviced and checked for proper functioning at regular intervals by specially trained craftsmen, preferably under a service contract;
- it is strongly recommended to install a diesel-engined power generator of sufficient capacity and with an automatic starting device, in order to overcome possible breakdowns in the electric power supply;
- it is recommended to store sufficient quantities of liquid nitrogen at all times to overcome possible breakdowns of the nitrogen producing plant itself.

### Liquid nitrogen containers

Liquid nitrogen containers differ in evaporation losses, depending on size, construction and quality of the insulation.
The best containers are made of stainless steel or aluminium; they have high-vacuum insulated double walls and are provided with highly efficient insulation materials.

Static holding times of frozen semen storage containers vary between 20 and 100 days, with evaporation rates of 5% down to 1% per day.
Containers with longer static holding are generally larger and not suitable for use in the field.

Small containers designed for field use need liquid nitrogen refills at regular intervals varying from 3 to 40 days depending on size and quality of the container in question.
Liquid nitrogen containers are complicated in construction. They are therefore liable to irreparable damage and should be handled with care.

The insulation capacity of containers diminishes with time, mainly due to loss of vacuum.

It is advisable to have some spare containers available for the immediate transfer of frozen semen in case of a sudden breakdown.

# 7 Heat detection & timing of insemination

## 7.1 Heat detection

Once more the topic ‘heat detection in cattle’, to underline the importance of this topic (see Book I); here specifically in the context of artificial insemination.

As inseminators have to rely on the observations of the farmer or his stockmen, the latter must be encouraged to obtain the highest possible degree of efficiency in heat detection.

Heat symptoms are:

1 *Pre-heat,* lasting 6 to 18 hours
- the cow is restless, alert, her ears are standing up, the eyes are bright and she sniffs the air,
- she licks and approaches other cows; sometimes she moos,
- the vulva is swollen and reddened.

However, not all cows show pre-heat symptoms and not all symptoms may be equally apparent.

2 *Standing heat,* lasting approximately 18 hours or less
- symptoms as mentioned above but more clearly noticeable,
- transparent mucus discharge from the vulva may be observed, mainly in the second part of the standing heat period; however, mucus discharge is a rather poor heat symptom as it can also sometimes be noticed in the midst of the sexual cycle and even in pregnant cows,
- there may be a drop in milk production
- but the MOST IMPORTANT and reliable sign of standing heat is that the cow invites and allows other cows to mount her.

3 *Post-heat,* lasting approximately 10 hours
- all the above heat signs become less apparent and disappear gradually,
- the cow does not allow other cows to mount her any more,
- in many cases some 12 to 48 hours after cessation of the standing heat period, a bloody discharge from the vulva can be noticed (ie. discharge ‘with a bloody tinge’).

Keep in mind that heat signs are not shown continuously.
During feeding and milking and during the hot periods of the day most cows do not show any heat symptoms at all.
Therefore the best time to observe heat symptoms is during the cooler periods of the day and when the animals are allowed to run free.

Cows which are expected to come into heat or could come into heat (cow calendar! refer to Chapter 11.3) should be observed closely, at least *three times a day,* during which especially the changes in behaviour should be watched.

So, a reliable heat detection is only possible in free running cattle. In permanently housed cattle the symptoms of standing heat cannot be detected at all. In such cases it is advisable to allow the cows to run free in a paddock or in a grazing pasture at least twice daily, under close observation. And this in the cooler periods of the day when the climate is hot (early in the morning and late in the afternoon). Allowing cattle to run free during the night could be most helpful for a better expression of heat symptoms; but of course at night a herd is difficult to observe properly.

**Bos taurus versus Zebu breeds**

Normally it is not difficult to detect heat symptoms of Bos taurus cattle in temperate climates.
In the tropics such cattle tend to show slightly shortened and less intense heat symptoms. Moreover, the symptoms are mainly expressed in the period between sunset and sunrise.

But with appropriate measures heat detection of these breeds in the tropics should not be too difficult. Missed heats are generally the result of *negligence* or *lack of knowledge* about heat symptoms and it is usually not the cow which is at fault.
Therefore herdsmen should be instructed how to detect heat symptoms *and* should be motivated to apply proper heat detection methods. In large dairy herds experience has shown that a bonus for any cow seen in heat improved the motivation of herdsmen to watch their cows closely and to record any animal seen in heat.

Many Zebu breeds, however, show heat symptoms less clearly and in most Zebu breeds the true standing heat period appears to be of much shorter duration than in Bos taurus breeds.

The use of vasectomized bulls in Zebu herds is often recommended in order to obtain accurate heat detection.
A cow coming on or going off heat can thus be spotted, because the bull is following her and attempts to mount her, particularly at the onset of true heat. But the cow will not stand for service unless she is actually in heat.
Vasectomized bulls are quite suitable for large herds but not for the peasant herd owner. However, heat detection is mainly a problem in larger herds; in smaller herds the cows are generally under closer observation.

In (large) herds any observed heat should be *recorded immediately,* in an appropriate administrative system.
Such a system makes it possible to predict the date of the next expected heat and this is of great help for proper heat detection. Moreover, it provides the necessary information for the veterinarian in case of breeding problems.

## 7.2 Timing of insemination

The best conception rates are obtained with inseminations done between 4 to 24 hours after the onset of heat. So, inseminations should be performed in the middle or latter half of the heat period or within 8 hours after cessation of the standing heat symptoms.

Inseminations performed within 4 hours after the onset of the standing heat period result in lowered conception rates.
Inseminations more than 8 hours after cessation of the standing heat symptoms also result in lower fertility; moreover, the risk of causing injury to the genital organs has increased (the uterus is more susceptible to inflammation and the cervix has usually tightly closed again by this time).
Bull semen cells are not capable of fertilisation immediately after insemination. It takes several hours in the cow’s genital tract before semen cells are capable of fertilising an egg (ovum).
In normal cows ovulation (ie. the release of an ovum) takes place some 10 hours after cessation of the standing heat symptoms. Unfertilised eggs can survive in the cow’s oviduct for a few hours only. Therefore inseminations at too short a time before ovulation result in lowered conception rates as the ovum can be dead already before the semen cells are capable of fertilisation.

On the other hand bull semen can not survive in the cow’s sexual organs for a period longer than approximately 24 hours. Therefore too early inseminations will result in the semen cells being dead before ovulation has taken place.

*As a general rule* it can be recommended that cows seen in heat early in the morning up to 8 a.m. should be inseminated on the same day .........
whilst cows coming in heat later on (that day) should be inseminated the following morning.

In practice, in view of the inevitable delay between the cow being seen in heat and the inseminator arriving at the farm, there is little chance of the insemination being done too early in the heat.
But this risk exists where inseminations are performed by herd owners themselves; here the tendency is to inseminate as soon as heat symptoms are detected.

Suspending inseminations for one day per week reduces the chances of conception in cows in heat on that particular day (and held over to the next day).

### Optimum time for insemination after calving

The uterus of a healthy, normal cow has returned to its normal size by about the 28th day after calving.
However, for optimum fertility a much longer interval between calving and insemination is necessary.
Fertility increases with time, up to 90 days after calving and levels out thereafter.

It is generally agreed that a minimum of *60 days* after calving is required to obtain acceptable conception rates, in the case of normal, healthy cows.
A diseased uterus presents a quite different picture in this respect (ie. the interval becomes much longer).

# 8 Insemination techniques

**Insemination must be done properly** and this is essential for any AI operation.
Inseminators must be trained in applying the correct techniques and thereafter they must be regularly checked by experienced supervisors on the correct application of these techniques.

Insemination with the use of a speculum is a method that has been widely applied but that has now been largely replaced by the *recto-vaginal technique*. The speculum method found favour because little experience was needed.
However, there were certain drawbacks. One of these was the need for cleaning and sterilizing the speculum after each insemination. Conception rates were not as good as they are with the recto-vaginal technique.
The recto-vaginal technique on the other hand requires considerable practice before proficiency is reached.
See Chapter 12, 'artificial insemination and rectal palpation'.

*Proper insemination techniques involve:*
- proper identification of the cow before insemination,
- interrogating the farmer in order to obtain a clear picture about the 'history' of the cow,
- careful approach and handling of the cow prior to and during insemination,
- external examination of the cow to be inseminated,
- proper selection of the semen which is required,
- application of correct thawing techniques where frozen semen is used,
- proper preparation of the insemination syringe,
- hygienic handling of syringe and cow,
- thorough rectal examination of vagina, cervix and uterus before actual insemination,
- proper introduction of the syringe into the cervix,
- deposition of the semen at the right spot in the genital tract of the cow,
- correct and legible filling in of the insemination documents after insemination,
- proper cleaning and handling of all materials and equipment,
- correct attitude of the inseminator towards the farmers or their personnel.

These points are elaborated below.

### Identification

Before insemination, the cow to be inseminated should be properly identified by checking her against the individual cow card which is presented by the farmer.

For every new cow to be inseminated, the inseminator has to hand over a cow card to the owner.

The cow card remains with the cow (ie. the owner).

**COW CARD**

| | |
|---|---|
| **OWNER** | Farm Number: Name:<br>Address: |
| **COW** | Ear Notch Number: Breed: |

| **YEAR** | 1st insemination | | | 2nd insemination | | | 3rd insemination | | | 4th insemination | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | date | number | bull | date | number | bull | date | number | bull | date | number | bull |
| | | | | | | | | | | | | |

*Figure 3: Cow card*

**Before the cow is inseminated**, the inseminator must ask for the cow card, in order to

- identify the cow to be inseminated
- find out whether it is a first or a repeat insemination
- find out whether the cow could be pregnant or not
- know the data of the previous insemination

For new cows to be inseminated the inseminator should make up an individual cow card prior to insemination.
This cow must show the required identification marks.
With spotted cows identification can be based on colour markings, but in evenly coloured cows tattooing, ear marks or ear notching is required for proper identification.
Marking of cows is easiest done with ear marking pliers. Where this system is adopted, every inseminator should be thoroughly familiar with the international ear notch code (see following page).

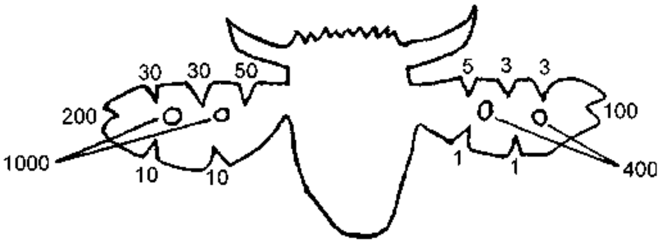


*Figure 4: The international ear notch code*

The individual cow card should be left with the cow and at every following insemination this cow card should be shown by the person who presents the cow.
The cow card provides valuable information:

- is it a first or a repeat insemination?
- in case of a repeat insemination, the length of the preceding sexual cycle;
- the number of previous calvings;
- the age of the cow;
- and possibly other information.

### The 'history' of the cow

Before starting with the actual insemination, the person presenting the cow (possibly the owner) should be asked about when the cow calved and whether any abnormal discharge or abnormal behaviour was noticed.
Cows should not be inseminated within 60 days after normal calving and not within 90 days in case of an abnormal calving or in case of abnormalities after calving.
Cows which have shown abnormal discharge from the vulva in the three-weeks period prior to present insemination should also be refused.

### External examination

The next thing to do is the external examination of the cow:

- the tail, the vulva and adjacent parts should be carefully checked for the presence of mucus or purulent discharge;
- are there signs of standing heat on the rump or the tailhead - a cow that has been mounted is likely to show mud or scars on these areas;
- open the lips of the vulva with the fingers and check the clitoris and the mucous membranes; in case of abnormal discharge, flocks of pus are likely to be detected on the clitoris;
- cows which show abnormalities in external examination should be refused for insemination and the farmer should be advised to consult a veterinarian.

### Selection of the semen

Semen containers should be opened only after the cow to be inseminated has been properly identified and examined.
In selecting the required semen in the container, take care not to warm up the semen in the container.

Liquid semen can be used immediately, but frozen semen must be thawed prior to insemination.

### Thawing of frozen semen

*Ampoules and straws* require thawing water of 35 °C. It is strongly recommended to use a thermos flask for carrying thawing water of correct temperature. The temperature of the thawing water should be carefully checked with a thermometer. If necessary, the temperature should be adjusted by adding either hot or cold water until the correct thawing temperature is reached.

*Pellets* are thawed in 1 ml of a special thawing solution at a temperature of 35 °C. The thawing solution should be packed in ampoules of 1 ml and carried in a thermos flask with water of exactly 35 °C.

A metal tweezer should be used to take a dose of frozen semen out of the liquid nitrogen container; the points should be cooled down in the liquid nitrogen for a few seconds before the semen dose is touched.
After the semen dose has been taken out of the container, it should be thawed immediately, as rapidly as possible.

Pellets are immediately transferred into 1 ml of the special thawing solution at 35 °C for at least 35 seconds; in this way optimum survival rates will be obtained.

Thawed semen must be used for insemination as soon as possible and in any case within one hour after thawing.
Once thawed, semen should never again be put back in liquid nitrogen as this will destroy the semen immediately.

### Preparing the syringe (also called 'pipette' or 'pistolet')

The syringe should have about the same temperature as the thawed semen. Syringes which are too cold or too hot can damage the semen.
If necessary, the temperature of a syringe can be raised by rubbing it with a clean piece of paper; or lowered by moving it in the air.
Sterile, disposable plastic syringes should be used for liquid semen, ampoules and pellets.
Special metal syringes are required for insemination of semen packed in straws; they are covered with a sterile, disposable plastic sheet prior to insemination.
The sterility of the syringe should be preserved throughout the whole operation; syringes should only be handled at the rear end to avoid contamination.
It is recommended to hold the loaded syringe between the teeth while the cow which must be inseminated is approached.

### Rectal examination of the cow

- One hand is gloved and the glove is lubricated with a special, non-irritating lubricant;
- approach the cow with the syringe kept in the mouth. Insert the gloved hand in the rectum with gentle movements (bending of the tail should be avoided as this might upset the cow);
- if necessary, remove the dung from the rectum with gentle hand movements;
- carefully palpate the cervix and the two uterine horns. If the cervix is soft and pliable and if the uterine horns are rigid and contract on palpation, the cow is likely to be in heat;
- however, if the cervix is hard and rigid and the uterine horns soft and flappy, it is unlikely that the cow is in heat;
- the two uterine horns should be carefully checked for endometritis, pregnancy or abnormalities;
- cows with abnormalities or without obvious heat signs should **never** be inseminated;
- *avoid touching the ovaries* during rectal palpation. Palpation of the ovaries of cows in heat can provoke rupture of the follicle and then the cow will no longer be able to conceive.

### Introduction of the syringe (after everything has been found to be in order)

- After the genital tract has been examined by rectal palpation, the arm in the rectum is withdrawn until only the hand remains in the rectum;
- clean the vulva with the free hand, with a dot of cotton wool, a towel or a piece of toilet paper. An inseminator should always carry cleaning material in his trouser pocket, at the side of his free, ungloved hand;
- after the vulva has been cleaned, apply gentle pressure with the palm of the hand which is in the rectum, in order to push down the vulva somewhat. This will open the vulva;

- only at this moment is the syringe taken out of the mouth. Insert the tip of the syringe into the vulva and in doing this avoid touching the gloved arm or the cow herself;
- push the gloved hand toward the cervix. At the same time gently force the tip of the syringe to follow this movement alongside the roof of the vagina;
- next the gloved hand takes hold of the cervix, closes the fornix with the fingers and gently massages the cervix over the tip of the syringe. This action is *difficult to perform* and requires a lot of experience.

Note that the syringe should *never be pushed into the cervix* as the cow could be badly damaged when this is done.
The syringe should always be kept parallel with the gloved arm in the rectum. The hand in the rectum is supposed to do the job.

### Deposition of the semen

In case of a *first* insemination the semen should be deposited just in front of the internal opening of the cervical canal in the uterus. The syringe should **not** enter the uterus itself as this can cause serious injury to the uterine wall.
In case of *repeat* insemination there is the possibility that the cow is actually pregnant. Therefore it is recommended to deposit the semen in repeat inseminations between the third and the fourth cervical ring (or fold) and **not** to pass the cervix completely.

All the semen should be deposited at the same site. Withdrawal of the syringe during the emptying should be avoided.

### Checks after insemination

- After insemination, withdraw the gloved hand from the rectum. With the same movement withdraw the syringe from the genital tract;
- then carefully check the tip of the syringe for the presence of pus or blood. If **pus** is detected at the tip of the syringe, there must be a purulent inflammation of the cervix or the uterus:
  - the farmer should be advised to consult a veterinarian
  - the inseminator himself/herself should improve his insemination techniques as referred to earlier, because this condition should have been discovered before performing the actual insemination

    If *blood* is detected at the tip of the syringe, this was caused by damage due to poor introduction techniques and the inseminator must improve his/her techniques.

### Gentle handling of the cow

In order to obtain good conception rates cows should be handled gently and in a friendly way. Cows must find the insemination pleasant; otherwise the semen will not be transported to the right place after insemination. The following helps in this respect:
- apply sufficient lubricant;
- avoid bending or touching the tail;
- nails should be clipped short, to avoid damage to the rectal wall;
- all movements in the rectum should be executed gently and carefully;
- do not apply force when inserting the syringe and when passing the cervical canal.

### Cleaning

After insemination, hands, boots and equipment should be cleaned carefully.

All insemination equipment should always be *impeccably clean* and stored in clean bags or boxes.

### Recording

After insemination fill in:

- the cow card;
- the insemination ticket (or insemination certificate); refer to following page.
- To be bound in booklets of 200 sheets, prenumbered in duplicate, and perforated at the top side.
- To be filled up with a sheet of carbon paper between the two sheets of a set.
- The first ticket to be handed over to the farmer, the copy ticket to be handed in at the main centre at least once a week.
- Every inseminator uses his own ticket book, so that the insemination number can be linked to the person who performed the insemination.

**INSEMINATION TICKET**

------------------------------ (perforation) ------------------------------

ARTIFICIAL INSEMINATION TICKET

Name inseminator: ......................
code number : ......................

| | | |
|---|---|---|
| FARM | Farm Number:<br>Address: | Name: |
| COW | Ear Notch Number: | Breed: |
| INSEMINATION | Date (year/month/day): | Bull: |
| PREVIOUS INSEMINATION | Date (year/month/day): | Insemination Number: |

RECEIPT I hereby declare that I have received the amount of M .......
for the insemination of the above-mentioned cow.

.................. ................................

(date) (signature)

*Figure 5: Insemination ticket*

# 9 Record keeping in an AI operation

An AI organisation must have an efficient record keeping system. The office of the main centre must have all the attributes of any medium-size thriving business concern.
It is not an exaggeration to say that *without efficient record keeping any AI programme is doomed to failure.*

One should not underestimate the amount of work involved in an efficient record keeping system.
Thousands of inseminations should be related to the end result, entered on farm record cards and used for the compilation of monthly statistics per area, per bull and per inseminator.
Non-return rates have to be calculated monthly, per bull and per inseminator.
The bull semen production unit must keep records of each semen collection, its quality, dilution rate, storage time, etc.
Lastly, financial records on money received, cash disbursements, etc.,require standard bookkeeping systems.

As a programme grows, it is wise to consider the use of **electronic data processing** for statistical compilation and analysis.
Although the initial investment may appear to be high, considerable time savings are possible and financial management is greatly simplified.
Modern data processing systems provide high processing power at moderate cost and may even be leased on a monthly or yearly basis.

In the following a simple record system is presented; it is designed for *hand operation.*
Its application can be recommended in small or medium-sized AI operations.
It is based on a system of **one** paid insemination followed by up to three repeat inseminations **and** on collection of insemination fees by the inseminators, but it can be adapted to even any other insemination system.

## 9.1 Example of a simple record keeping system

The inseminator must fill in **two** documents for each insemination namely the *insemination ticket* in duplicate and the *cow card.*
Moreover, he must cash the insemination fee which is due.

### The insemination ticket

The inseminator must fill in an insemination ticket for **every** insemination.
An example of an insemination ticket is presented on page 42.
The insemination tickets are pre-numbered in duplicate:
- one copy is handed over to the farmer and serves as a receipt
- the other copy is handed in to the main centre; this is done at least once a week

The total number of straws used by the inseminator should be the same as the number of tickets handed in at the main centre.

In case a mistake is made in filling in the ticket, the inseminator must cross the ticket and must hand in **both** copies to the main centre.

Sometimes straws are thawed without being used for an insemination. Such straws must be kept and handed in to the main centre as so-called ‘unused straws’.

It is important that every inseminator uses his own pre-numbered ticket book and that for every inseminator the ticket numbers are different. Thus every insemination can be traced back to the inseminator who performed it, by its insemination number.

In case an insemination is performed in a new herd, the number of the farm is not known. In that case the inseminator leaves the space after ‘Farm Number’ open and he writes on the ticket ‘New Farm’. Upon receipt of such a ticket in the main centre this farm can be given a number.

### The cow card

The inseminator always carries a sufficient number of cow cards with him. For every new, not previously inseminated cow, a cow card is filled in; if necessary, the cow concerned is identified by ear notching, by the inseminator.
An example of a cow card is given on page 36.
The cow card remains with the cow and must be presented by the farmer to the inseminator.
The inseminator must check the cow card before inseminating the animal; it gives important information to the inseminator:

- is it a first or a repeat insemination
- the length of the preceding sexual cycle (in case of a repeat insemination)
- the age of the cow
- the number of previous calvings

Moreover, the cow card is the only means to identify the cow properly before insemination.

**Remember: no insemination without prior identification!**

### Transfer to the main centre

At least once a week all subcentres must hand in

- the insemination tickets
- the insemination fees which have been collected
- the ‘unused straws’

The main centre issues receipts to the subcentres. Each receipt must be countersigned by the senior inseminator of the subcentre concerned.
A duplicate of the receipt is given on page 50.

### Semen stock inventory

The main centre keeps a semen inventory book (on page 51).

The number of insemination tickets received plus the number of ‘unused straws’ from a subcentre must be the same as the number of straws issued to this subcentre minus the semen stock in the subcentre.

The semen stock in a subcentre should regularly be checked by the main centre.

It is advisable to keep the semen stock in the subcentres as small as possible in order to facilitate the administration.

The amount of ‘unused straws’ should be kept as low as possible.
Inseminators who spoil relative large amounts of straws must be warned. They should thaw the straws more carefully and should more consciously select and identify the cows **before** insemination.

### Membership registration

The main centre keeps a register of membership.

All farms that make use of AI are provided with a farm number.
Make an entry in the register every time when a ticket is received with the mention ‘new farm’; for this farm a new number should be issued.

It is advisable to coordinate the register of membership with that of other dairy organisations (if existing) so that all organisations work with the same farm number for the same farm.

A copy of the register of membership must be available at each subcentre and these copies must be kept up to date.

### Financial administration

The total amount of insemination fees to be received from the subcentres can be calculated from the insemination tickets received.

Fees are cashed by the main centre against issue of a receipt countersigned by the subcentre concerned.
This receipt is made up in duplicate; one copy is issued to the subcentre and the other is kept at the main office.

The main centre keeps book on all incomes per bull and per inseminator.

### Bull and inseminator statistics

The main office keeps
- one bull book for every bull in use
- one inseminator book for every inseminator employed

Examples are given on pages 51 and 52.
The directions for use of these books are as follows.

Upon receipt of the insemination tickets, these are first assorted according to insemination number.
Thereafter all insemination tickets are entered in the respective inseminator books.
One line is used for each insemination.

In case of a **repeat** insemination, the number of the preceding insemination is **underlined**.

After that the tickets are assorted per bull and within bulls in numerical sequence.
Now the information of the tickets can be entered in the respective bull books.
Here again, for each repeat insemination the number of the preceding insemination should be **underlined**.

In case this preceding insemination was a first insemination, in the column under ‘number’ a **2** is entered. A **3** is entered in the column under ‘number’ where the preceding insemination was a second insemination and so on.
This is a check on the correct cashing of insemination fees by the inseminators.
Where a **4th** insemination is registered, the inseminator must have cashed and handed over an insemination fee.

Information about the results of P.D.’s (pregnancy diagnoses) must also be entered (in the bull book as well as in the inseminator book), as soon as it becomes available.

One page must be used (if necessary one double page) for each month. This holds for the inseminator books as well as for the bull books.

### Farm record cards

Finally the insemination tickets are assorted according to farm number and entered on farm record cards.
An example of a farm record card is given on page 54.

These cards are useful; for instance when a farmer reports too many repeat inseminations (or other problems), or when incorrectly completed AI certificates must be adjusted.

They can also help veterinarians making pregnancy diagnoses or investigating fertility problems.
In areas where milk recording systems exist, copies of the farm records should be sent to the milk recording office at least twice yearly, for pedigree registration of newborn calves.

# 10 Calculation of fertility rates

Calculation of fertility rates IS A MUST. It is done in order

- to check and to supervise the quality of the work done by the individual inseminator
- to check the semen quality of the individual bull

The *only absolute way* in which fertility from artificial insemination can be assessed is from the number of calves born. But even this does not give a true measurement of conception unless it takes into account the number of calves which are aborted.

Making enquiries at about the expected date of calving (some 280 to 300 days after the last insemination) can give valuable information about the productivity of the AI service in terms of number of calves produced.
It also provides very useful information about the average gestation period, the average birth weight, the occurrence of calving difficulties, the number of stillborn calves and inborn defects at birth; this can be related to the bulls which have been used.

However, the above method cannot be used to monitor the conception rates of sires and inseminators (so to speak), because the interval between insemination and calculation of fertility rates is over nine months ....... much too long to take adequate action in case of low fertility rates!!

Therefore methods of making earlier estimations of fertility rates have been developed.
One obvious method would be *pregnancy diagnosing* all inseminated cows some six to twelve weeks after the last insemination. But this is expensive and time-consuming and experience has shown that it is often difficult to get full cooperation of all farmers needed to check all inseminated cows on pregnancy.

Hence most AI organisations calculate conception rates as *non-return rates from first insemination:*

- the 30-60 days non-return percentage (so-called % 30-60 NR) is the percentage of cows and heifers inseminated for the first time that were **not** reported as requiring a repeat insemination at the end of the month following that in which they were inseminated;
- the 60-90 days non-return percentage is the percentage of cows **not** reported in heat at the end of the **second** month following that in which the first insemination was performed.

Note that calculation of non-return rates is **not** an exact measure of fertility; other factors may be involved such as the failure of the farmer to request a repeat insemination if the first insemination was not successful.

The average difference between the % 30-60 NR and actual pregnancy rate is approximately
20%; that between 60-90 NR and actual pregnancy rate 10%.

### Actual calculation

At the end of each month, as soon as all inseminations of the month in question have been entered, in the inseminator books as well as in the bull books, calculate the fertility rates *before entering new tickets* from inseminations performed in the next month.
As follows.

1 Count *all* inseminations performed during the month, in the bull book as well as in the inseminator books
   - the number calculated from the combined bull books should be the same as the number calculated from the combined inseminator books (a check).

2 Count all *first* inseminations performed during the month, in the bull books as well as in the inseminator books
  - ➢ the number obtained by summing up the countings of the inseminator books should be the same as the number obtained by summing up the countings of the bull books.

3 Count in each book *all underlined first* insemination numbers in the *preceding* month. So, for instance at the end of July, count all underlined first insemination numbers from June
  - ➢ the difference between the number of *all* first inseminations performed in one month and the number of *underlined* first inseminations in the same month provides (when calculated) at the end of the next month the number of 30-60 days non-returns
  - ➢ the % 30-60 NR can then be calculated by dividing this number of non-returns by the number of first inseminations in the same month and multiplying the quotient by 100.

4 Count in each book all *underlined first* inseminations from two months ago. So, for instance at the end of July, count all underlined first inseminations from May
  - ➢ the difference between the number of **all** first inseminations performed in one month and the number of *underlined* first inseminations in the same month provides (when calculated) at the end of the second following month the number of 60-90 days non- returns
  - ➢ the % 60-90 NR is calculated by dividing this number of non-returns by the number of first inseminations in the same month and multiplying it by 100.

5 Count in each book the number of P.D.'s after *first inseminations* performed in the month in question **and** the number of cows confirmed to be pregnant after P.D.
  - ➢ the number of pregnancies *after first inseminations* divided by the number of P.D.'s *after first inseminations* and multiplied by 100 provides the *conception rate.*

**Keep in mind:** fertility rates as well as conception rates are only calculated from **first** inseminations.

### Monthly statistics

At the end of each month a report should be made on each bull and on each inseminator.
Examples of such monthly reports are given on pages 55 and 56.

The figures on the bottom line of the monthly bull report (the overall total) should be the same as those of the monthly inseminator report.

A copy of the monthly bull report should be sent to the producers of the bull semen.
In case one of the AI used bulls shows fertility rates that are significantly lower than the mean fertility rate of the month in question, the head of the bull semen production centre should be contacted. He should take measures either to improve the semen quality of the bull under question or to replace it by semen from a more fertile bull.

Poor fertility rates of inseminators **can** be due to the fact that the semen used is from bulls which are not very fertile.
If this can be ruled out (because colleagues obtained good results) measures should be taken to improve insemination techniques of these inseminators.

Finally, **overall monthly statistics** are prepared (see page 57). They serve for management appraisal and improvement.

The management of an AI operation should aim at:
- ➢ an increase of the number of first inseminations
- ➢ an increase of the % 60-90 NR
- ➢ a decrease of the $LN_2$ consumption per insemination
- ➢ a decrease of the distance covered per insemination (kms)
- ➢ an increase in income from insemination fees
- ➢ a decrease of the number of straws used per first insemination

- To be made up in duplicate.
- One copy to be handed over to the inseminator at presentation of his tickets and unused straws, the other copy to be held in stock.

**TO SUM UP:**
**THE BEST POSSIBLE SERVICE**
**AT THE LOWEST POSSIBLE COST !!!**

**RECEIPT FOR TICKETS AND FEES**

---

**RECEIPT**

Received from inseminator ......... .......................................
(Number) (Name)

....... tickets, from No. ........... to No............., all included
(number)

and............... unused straws
(number)

SPECIFICATION

---

| | | | |
|---|---|---|---|
| ...... | tickets 1st at | .... = .... | .... straws bull .... |
| ...... | tickets 1st at | .... = .... | .... straws bull .... |
| ...... | tickets repeat at | .... = .... | .... straws bull .... |
| ...... | tickets repeat no fee | | ——— + |
| ...... | tickets fault | | ...... straws unused |
| ——— + | | ——— + | |
| ...... | tickets | ........ (money) | |

---

DATE: ........................ Signature: ...............

COUNTERSIGN INSEMINATOR: .......................................................

---

* To be made up in duplicate.

* One copy to be handed over to the inseminator at presentation of his tickets and unused straws, the other copy to be held in stock.

*Figure 6: Receipt for tickets and fees*

**SEMEN INVENTORY BOOK**

**BULL:**

| DATE | NUMBER OF STRAWS | | | | |
|---|---|---|---|---|---|
| | RECEIVED | FROM | ISSUED | TO | BALANCE IN STOCK |
| | | | | | |

*Figure 7: Semen inventory book*

**BULL BOOK**

Note: - <u>one</u> book per bull
- <u>one</u> (double) page per month

BULL: MONTH:

| DATE | Ins.No. | | | P.D. | | DATE | Ins.No. | | | P.D. | | DATE | Ins.No. | | | P.D. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1st | repeat | No. | date | +/- | | 1st | repeat | No. | date | +/- | | 1st | repeat | No. | date | +/- |

Ins.No. = insemination number
P.D. = pregnancy diagnosis

*Figure 8: Bull book*

**INSEMINATOR BOOK**

Note: - one book per inseminator
- one (double) page per month

INSEMINATOR NAME:
No. :

MONTH:

| DATE | Ins.No. | | BULL | P.D. | | DATE | Ins.No. | | BULL | P.D. | | DATE | Ins.No. | | BULL | P.D. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1st | repeat | | date | +/- | | 1st | repeat | | date | +/- | | 1st | repeat | | date |

*Figure 9: Inseminator book*

**FARM RECORD CARD**

THE ______________________ ARTIFICIAL BREEDING ASSOCIATION

| REGISTRATION AND EAR TAG NUMBER OF COW | DATE BRED | BULL | DATE RE-BRED | BULL | DATE RE-BRED | BULL | DATE RE-BRED | BULL | REMARKS | MEMS. PAID | ASS 'T. FWD | SERVICE 1st | SERVICE 4th |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | |
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| | | | | | | | | | | | | | |

DATE JOINED | BREED | D.H.I.A. | SIZE OF HERD FEMALES OVER 6 MO. | STERILITY DISEASE?

LOCATION | PHONE

OWNERS NAME | ADDRESS

FOR THE FISCAL YEAR ENDING ______________ 19 ___

*Figure 10: Farm record card*

**MONTHLY REPORT PER BULL**

MONTH:

| BREED | BULL | NO. OF IN-SEMINATIONS IN MONTH | NO. OF FIRST IN-SEMINATIONS IN MONTH | FERTILITY RATES | | CONCEPTION RATE | | SEMEN STOCK FLOW | | | MONEY CASHED |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | % 30-60 d. N.R. 1 MONTH AGO | % 60-90 d. N.R. 2 MONTHS AGO | NO. OF P.D.'s AFTER FIRST INS. | % OF POSITIVE P.D.'s AFTER 1st INS. | RECEIVED IN MONTH | ISSUED IN MONTH | SEMEN STOCK PER END OF MONTH | |
| | | | | | | | | | | | |
| BREED SUBTOTAL | | | | | | | | | | | |
| | | | | | | | | | | | |
| BREED SUBTOTAL | | | | | | | | | | | |
| | | | | | | | | | | | |
| OVERALL TOTAL | | | | | | | | | | | |

*Figure 11: Monthly report per bull*

**MONTHLY REPORT PER INSEMINATOR**

MONTH: ..................

| DISTRICT | INSEMI-NATOR NO. | NUMBER OF INSEMINATIONS PERFORMED IN MONTH: | | | | | | FERTILITY RATES | | CONCEPTION RATES | | MONEY CASHED | KMS COVERED |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1st | 2nd | 3rd | 4th | 4th | total | % 30-60 d. N.R. 1 month ago | % 60-90 d. N.R. 2 months ago | No. of P.D.'s after 1st ins. | % of positive P.D.'s after 1st ins. | | |
| | | | | | | | | | | | | | |
| DISTRICT SUBTOTAL | | | | | | | | | | | | | |
| | | | | | | | | | | | | | |
| DISTRICT SUBTOTAL | | | | | | | | | | | | | |
| | | | | | | | | | | | | | |
| OVERALL TOTAL | | | | | | | | | | | | | |

*Figure 12: Monthly report per inseminator*

## MONTHLY STATISTICS

**MONTH: ........................**

**A. PER DISTRICT**

| **DISTRICT** | | | | | | **TOTAL** |
|---|---|---|---|---|---|---|
| Number of inseminations | | | | | | |
| Number of 1st inseminations | | | | | | |
| % of total | | | | | | |
| % 60-90 days NR | | | | | | |
| Litres $LN_2$ per insemination | | | | | | |
| Kilometres per insemination | | | | | | |
| Total amount cashed | | | | | | |
| Number of straws per 1st insemination | | | | | | |

**B. PER BREED**

| **BREED** | | | | | | **TOTAL** |
|---|---|---|---|---|---|---|
| Number of bulls | | | | | | |
| Number of inseminations | | | | | | |
| Number of 1st inseminations | | | | | | |
| % of total | | | | | | |
| % 60-90 days NR | | | | | | |
| Number of doses received | | | | | | |
| Number of doses issued | | | | | | |
| Semen stock | | | | | | |
| Number of doses used per 1st insemination | | | | | | |

*Figure 13: Monthly statistics*

# 11 Miscellaneous

## 11.1 Training of inseminators

It is very important that inseminators receive a thorough theoretical and practical training before they are allowed to inseminate cows in the field.
In many countries there are special training facilities for inseminators.

The practical work should be organised and supervised by qualified and experienced instructors.
A sufficient number of cows of different breeds (if possible) and of different age should be readily available so that the trainee-inseminators have every opportunity to practise the skills which must be acquired during the training course.

Practical training is best done in *slaughterhouses:*

- unexperienced trainee-inseminators can cause serious damage to the genital organs of cows in trying to inseminate these cows;
- slaughterhouses provide the possibility to examine cows after slaughtering for internal damage caused by insemination;
- if colour dyes have been used for insemination, it is possible to check whether the 'semen' has been deposited in the right spot in the genital tract of the cow.

Methylene-blue solution in agar is widely used for this purpose.
The use of a specially designed battery-operated searing syringe has the advantage that even in cows slaughtered up to 48 hours after insemination the site of deposition of the 'semen' can still be detected.
When only a colour dye is used, the cows must be slaughtered as soon as possible because the dye tends to spread throughout the cervix and uterus rather rapidly.

Only **thoroughly trained** inseminators, capable of depositing the semen at the right spot without damaging the cow should be allowed to perform inseminations in the field.

The practical training (in the slaughterhouse) should be accompanied by theory lessons on reproductive anatomy and physiology, semen handling techniques, hygiene and disease prevention, with demonstrations and exercises wherever necessary.
Moreover, the trainees should learn the basic facts about dairy farm management, cattle genetics and breeding.

Although not strictly necessary it can be recommended that trainees learn to detect gross abnormalities of the sexual organs of the cow and to carry out pregnancy diagnoses.

The work done by inseminators in the field MUST BE MONITORED CONTINUOUSLY and INDIVIDUALLY (how is his/her 'conception rate'?). Those showing poor results must either be retrained or replaced.

Many AI organisations provide regular practical and theoretical refresher courses for their inseminators. This is a very good way to ensure optimum conception rates.

## 11.2 Artificial insemination in buffaloes

The domestic buffalo (Bos bubalis) is an important dairy animal in many parts of Asia (apart from being a draught animal). Also in the Middle East (especially Egypt, Iraq and Turkey) buffaloes contribute significantly to dairy production.

What are the differences as far as reproduction and artificial insemination are concerned:

1 The fertility of buffaloes is definitely lower than that of cattle.
  Buffaloes reach sexual maturity at least six months later than cattle and also the calving interval of buffaloes is significantly longer than that of cows.
2 The male buffalo is more easily trained for artificial insemination than the bull.
  Many buffaloes will ejaculate into the artificial vagina at the first attempt.
  The buffalo is also less discriminating with respect to a teaser and does not require as much pre-collection stimulation.
  The thrust given by a male buffalo at the time of service is much less forceful than that of the bull.
  Artificial vaginas for buffaloes should have a lower temperature than those used for bulls; the optimum temperature is 39 to 41 °C.
3 The volume and concentration of the semen obtained from male buffaloes is less than that obtained from bulls and there is relatively much seasonal variation.
4 Buffalo male cells can readily be distinguished from bull cells by the shape of the heads of the cells. Buffalo male cells rather look like those of the ram (male sheep) in outline, but buffalo cells are shorter and narrower.
5 Nonetheless buffalo semen can be processed in exactly the same way as bull semen.
6 Insemination of the female buffalo can be done by the recto-vaginal technique as in the cow. However, manipulations through the rectal wall must be carried out more gently and more carefully than in the cow because the capillaries in the rectal wall of the buffalo appear to be more fragile and they tend to bleed readily.
7 The heat tolerance of the buffalo bulls is lower; therefore particular attention must be given to shelter requirements of buffalo bulls kept in studs.
  During hot summer months the bulls should have free access to clean wallows or, if that is not possible, should be given cool showers or splashed with cool water daily.
8 Special care must be taken to prevent fighting between buffalo bulls; they are inclined to be more aggressive than cattle.
  Dehorning of buffalo bulls is seldom practised because the horn is greatly valued as a breed characteristic.

## 11.3 Aids to heat detection

An **effective recording system** is the most important aid to heat detection.
It may begin with a small notebook carried ‘in the field’, to make notes of cows seen in heat.
Every heat should be noted down, even if the cow in question is not due to be served or inseminated. In this way it is possible to predict the date of the next heat so that the cow can be watched carefully at that time. Conversely, if a cow is suspected to be in heat, if there is a record of the previous heat (about 21 days ago), this will help to establish the fact that the cow is in heat.
This ‘basic field notebook’ necessary with larger dairy herds must be followed up by a proper recording system at home or in a suitable place at the farm.

A simple and effective aid is a so-called *21-day-calendar,* as shown below. For the year 1999, 25,26 and 27 January 66 21 days later is 15, 16 and 17 February 66 21 days later is 8,9 and 10 March, and so on.

| January | February | March | and so on. |
|---|---|---|---|
| ....... | ........ | ....... | |
| ....... | ........ | ....... | |
| 25 | 15 | 8 | |
| 26 **X** | 16 **X** | 9 | |
| 27 | 17 | 10 | |
| ....... | ........ | ....... | |
| ....... | ........ | ....... | |

This calendar shows at a glance that cow X recorded in heat on 26th January, is due again on 16th February.

A more sophisticated aid is the **disk model cow calendar.** It is also called 'breeding wheel'.
It can be bought internationally; there are various models. It can also be home-made, in a carpenter's shop. It should be installed near the cows: farm office, dairy room, a store or any other convenient place. For a picture, see page 10 of the STOAS publication **'Guide to Effective Teaching'.**

The following is a description of a disk model cow calendar with a revolving disk and fixed indicators.
See illustration on page 63.

The disk is divided into 12 sections. Each section represents one month and is sub-divided into days.

The fixed indicators indicate successively:
- the insemination date (or date of natural service),
- the date on which pregnancy has to be checked (21 days after the insemination date),
- the date of drying off (seven months after the insemination date),
- the (expected) calving date (nine months after the insemination date),
- sometimes a second pregnancy check indicator is used (six weeks after the insemination date). The disk is further divided into four or five circles, from its centre. These circles serve to indicate first, second, third, etc., insemination/natural service.

Normally the *insemination date is the starting date*. Every day the disk is turned by one day.

The cows are represented by their names or by their numbers, which can be moved and stuck on the disk, one way or another.

### How to proceed

*Insemination*
Today is April 21st (assumption). The cow which is on heat today is inseminated and its number is placed under the insemination indicator, in the outer circle.
Three weeks later (May 12th), the number of this cow has come under the (first) pregnancy check indicator.
If the cow comes on heat again, she is again inseminated and her number is again placed under the insemination indicator (May 12th). The number is now placed on the second circle, to indicate that it is a second insemination.
Some cows come back after six weeks, hence the second pregnancy check indicator.

*Drying off*
The cow which has been inseminated on April 21st, arrives under the drying off indicator on November 21st. The cow should then be dried off.

*Calving*
Two months after drying off the cow arrives under the calving indicator. In case actual calving takes place before or after the expected calving date, on the actual calving date the number is placed under the calving indicator.

*After calving*
When the cow comes on heat for the first time after calving, it is usually too early to inseminate. Depending on the date and the desired calving interval, the number is placed 1, 2 or 3 periods of three weeks before the insemination indicator.

*Daily use*
One glance at the calendar will give the farmer (or the person who is responsible) sufficient information. Only cows whose numbers are under (or near) an indicator, need attention as far as insemination, pregnancy checking, drying off and calving is concerned.

Finally, the disk model cow calendar is a relatively sophisticated tool. Quite often, especially on the smaller dairy farms, a *simple card system,* with a card for every individual cow, will be more appropriate.

The next page shows an illustration of the disk model cow calendar.

Other aids to heat detection in dairy herds are:
1 heat mount detector
2 closed circuit television
3 a teaser bull (vasectomized; see page 32)
4 movement detector
5 temperature measurement

The above aids to heat detection (with the exeception of **3**) are associated with technically advanced ('computerized') dairy enterprises.

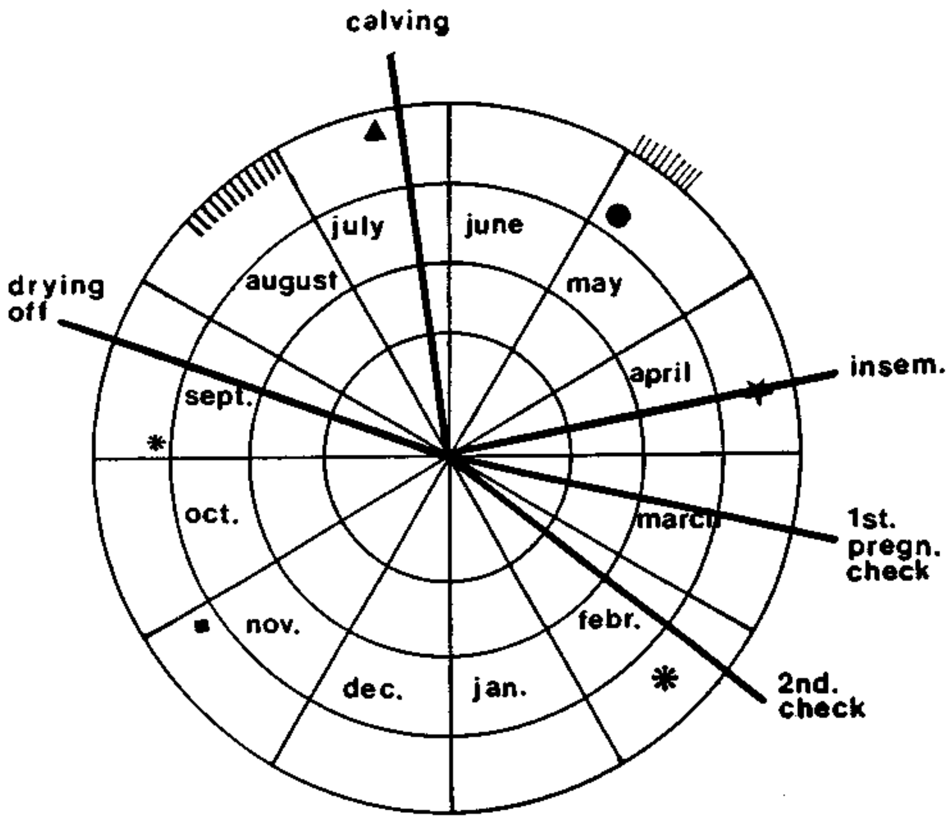


*Figure 14: Disk model cow calendar*

# 12 Artificial insemination & rectal palpation

Also refer to Chapter 8 of this book.

To enter the rectum, the fingers of the hand must be formed into a cone. Then the hand must gently but firmly be passed through the anus of the cow.
The entry usually elicits a defecation reflex; in any case the rectum must be emptied before the examination can proceed. Do not forcefully manipulate against the peristaltic waves or against a rectum ballooned with air, as this usually results in trauma as well as inadequate examination.

Should the rectum fill with air, reach forward and gently grasp the first contracted ring of rectal mucosa, then slowly pull backwards. This will usually stimulate a peristaltic wave which will evacuate the rectum.

The 'landmarks' of greatest value in rectal examination are the *cervix,* the shaft of the *ilium* (hipbone) and the *pubis* (pelvic brim).
The first thing to do is to locate the pelvic brim; if one then sweeps the cupped hand along the brim, the cervix may be located as a firm, cylindrical structure in the midline or just over the pelvic brim.

To pass the insemination pipette (other names are syringe or pistolet) successfully, one must be able to immobilise and control the cervix and guide the tip of the pipette with the hand which is in the rectum.

In addition, *strict sanitary precautions* must be observed; the introduction of faecal bacteria into the uterus must be avoided at all cost!!

**Proceed as follows:**
Insert the hand into the rectum. Clean the lips of the vulva with paper tissue.
In order not to contaminate the insemination pipette, the vulvar lips must be parted; this can be achieved by *one* of the following methods:

- place the arm in the rectum and lift the arm up sideways; this separates and opens the lips of the vulva,
- place the hand just inside the rectum with the thumb down; by spreading the thumb and then retracting the hand with thumb pressure against the rectum and upper cleft of the vulva, the vulvar lips will part,
- if the above methods fail, a wad of tissue can be worked between the lips of the vulva and be left in the ventral cleft.

Then pass the pipette into the vulva for about 5 to 7 cm, first upwards at a 60 °angle, then tilted to the horizontal.
Grasp the cervix at its vaginal end and push it forward. This manipulation prevents folds of the vagina from interfering with the passage of the pipette through the vagina to the external cervical os.

Loosely grasp the cervix and immobilise it against the floor or side of the pelvis. While holding the cervix in position with the thumb and forefinger, guide the pipette into the external os with the little finger.

The entry into the cervix can be checked by encompassing the cervix with the hand; if the pipette cannot be felt in the fornix one can be sure that it has penetrated into the cervix. Passage is then completed by manipulating the cervix in the horizontal and vertical planes while exerting **gentle** pressure on the pipette.

One can be certain that the pipette has passed through the internal os when its tip can be felt through the uterine wall.

In *no case* should the pipette be passed more than two cm beyond the internal os!!

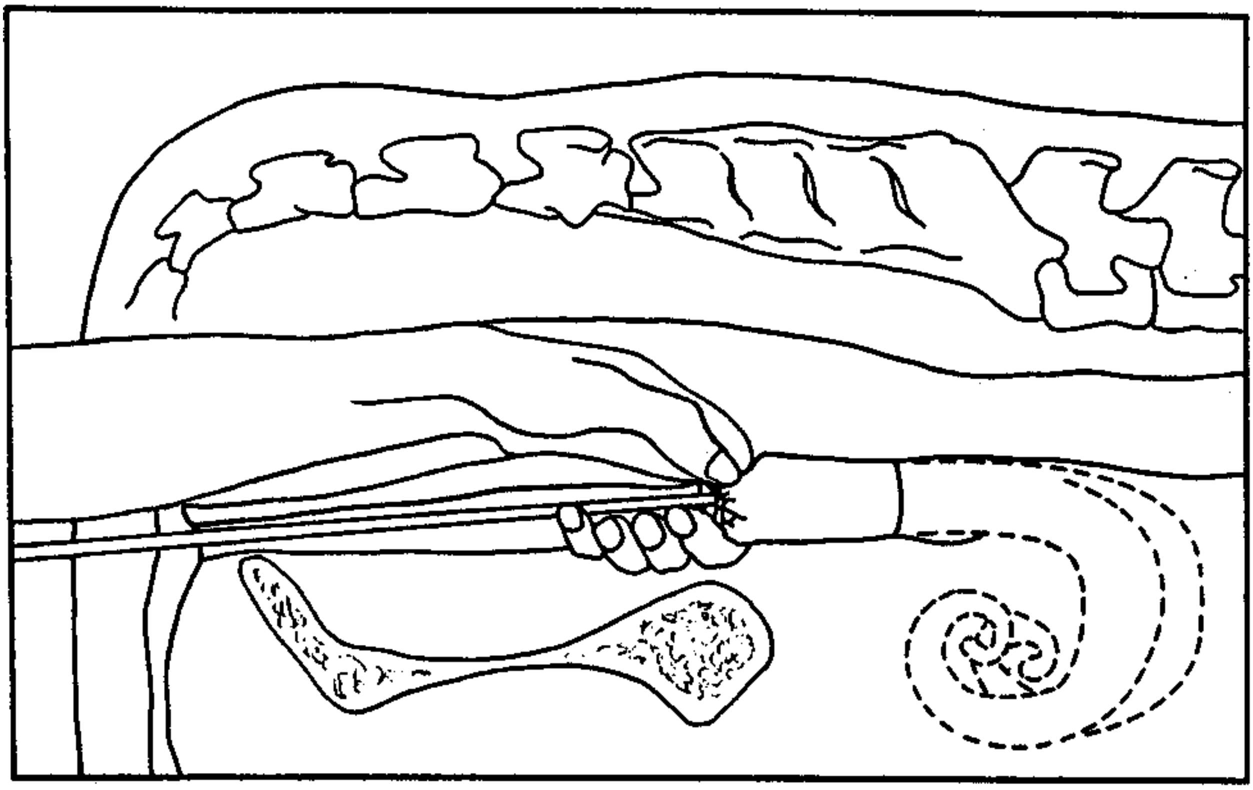

*Figure 15: Manipulating insemination pipette into cervix (adapted from M.H. Bartlett)*

Points to remember:

- Do not pull the pipette out of the vagina and then reinsert it; this will only carry contamination further up in the vagina.
- The pipette must not be withdrawn if the cow urinates or defaecates with the pipette well inserted into the vagina. In this case attempt to minimise faecal contamination by moving the pipette to one side, to allow the faeces to fall to the ground without contacting the pipette.
- Do not attempt to guide the pipette by violent manipulation of the end which is outside the cow.
- Do not force the pipette through the cervix as this can produce a cervical abscess or perforation of the uterus.
- The uterus is a bacteriologically sterile organ: do not allow the pipette to contaminate it!

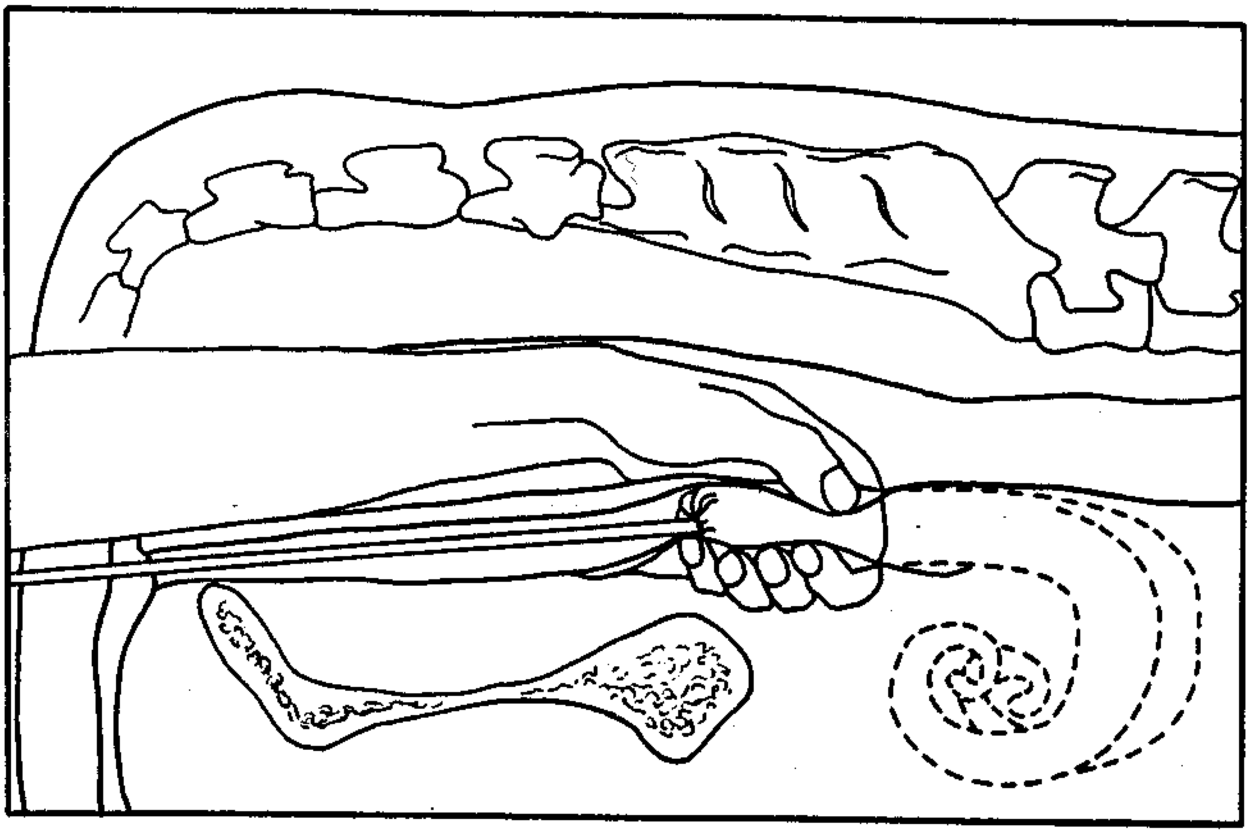

*Figure 16: Improper manipulation of cervix to introduce pipette*

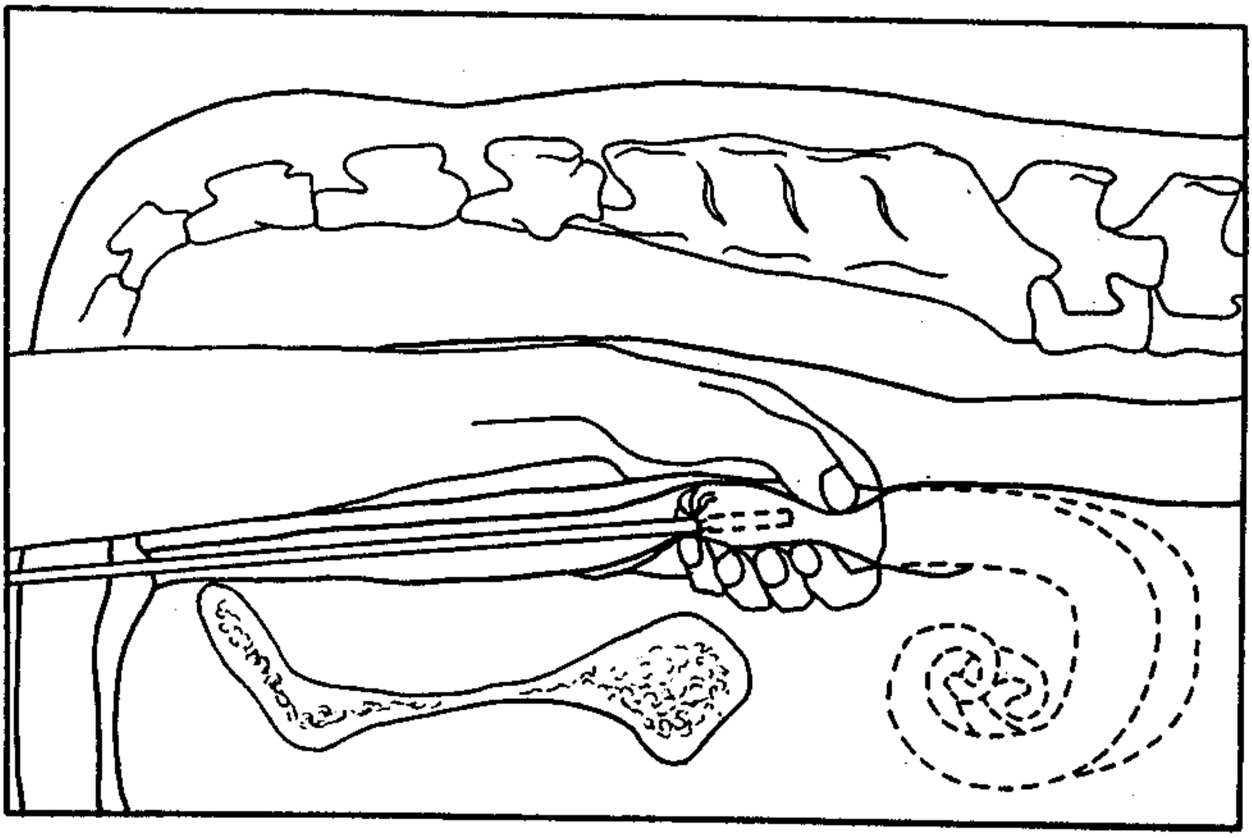

*Figure 17: Manipulation of pipette through the cervix*

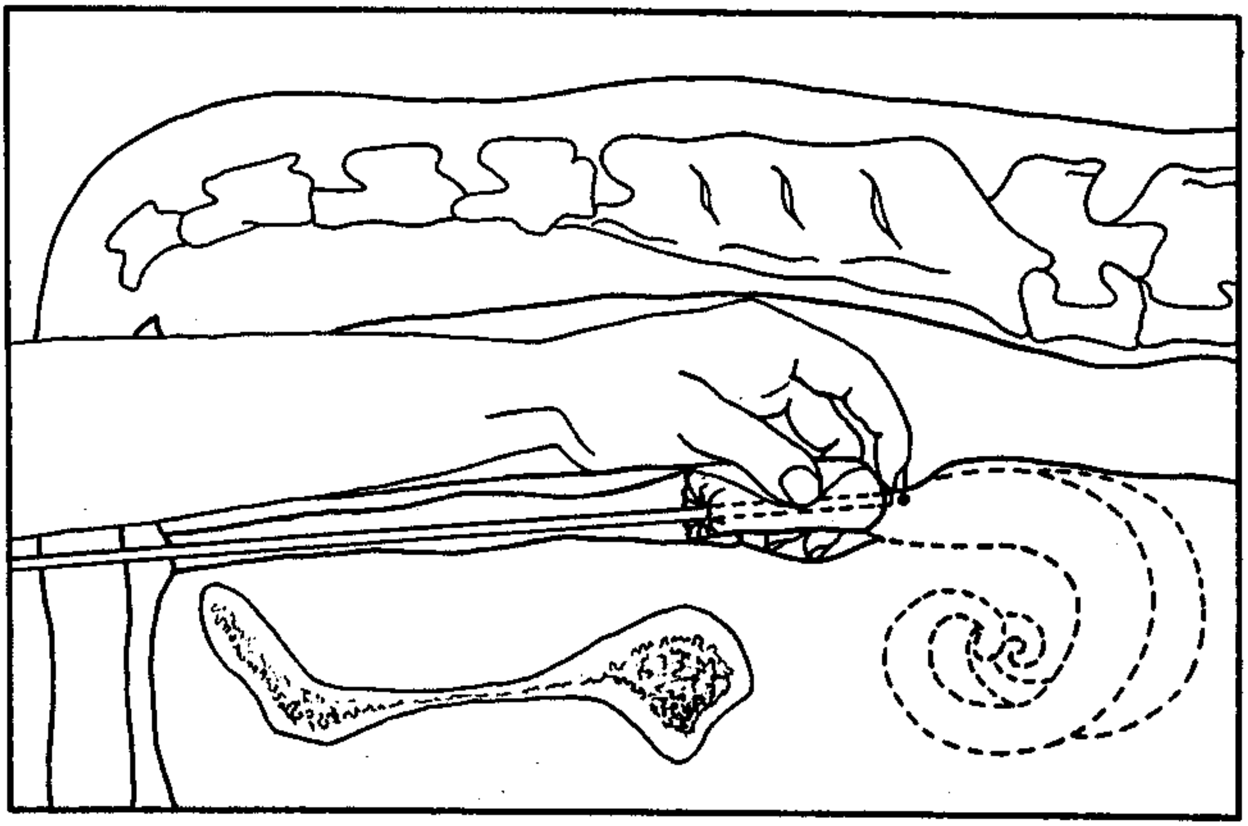

*Figure 18: Proper site for semen deposition*

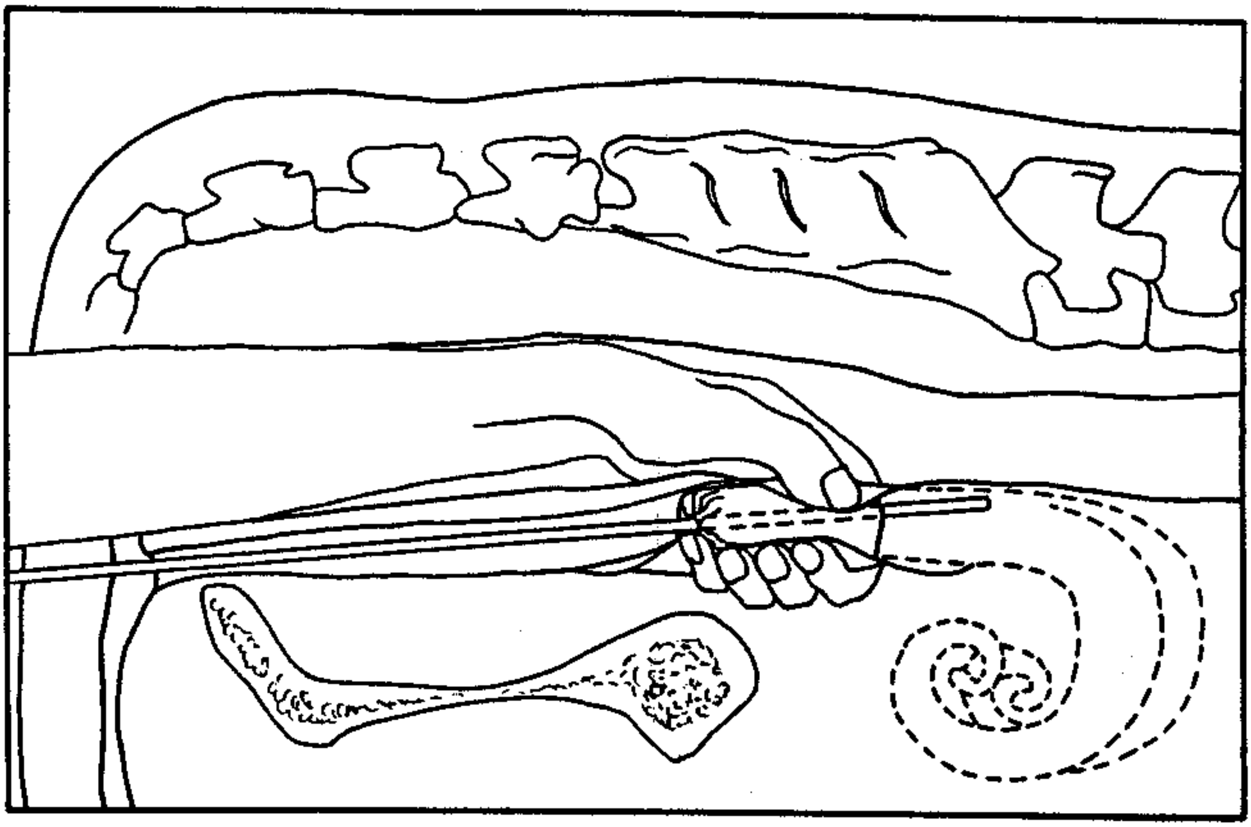

*Figure 19: Insemination pipette too deep in uterus*

# 13 Pregnancy diagnosis

Pregnancy diagnosis is also called ‘pregnancy determination’. It is usually shortened as PD.

There are three reasons why PD is important:
1 With PD we can identify and combat fertility problems in herds or individual cows at as early a stage as possible.
2 With PD we can identify non-pregnant cows and they can then be culled if desirable. In this way PD assists economy and efficiency.
3 With PD we can certify that a cow is pregnant; this is important where cows are bought or sold.

In a laboratory it is possible to measure the progesterone level in the milk of a cow in order to determine pregnancy at an early stage, say 21 to 24 days after service. Progesterone levels in milk (or blood) are indicators of corpus luteum function. A low progesterone level means that the cow is not pregnant.
This way of doing a PD requires a suitable laboratory and high quality management on the farm to ensure that samples are taken at the right time and are properly conserved and labelled.
Measuring the progesterone level in milk is not the only laboratory method that is available for PD.

In this guide we will only describe PD by means of the *rectal palpation* method. This remains the method of choice for pregnancy diagnosis in cows and can be applied anywhere.

PD by means of rectal palpation is an art. Like any art, it requires considerable guidance before it is mastered and then more or less *constant practice* in order to maintain proficiency.

Other requirements:
1 every effort must be made to avoid spreading infectious diseases from cow to cow or from farm to farm; suitable protective clothing , including plastic or rubber arm-length gloves, must be worn and kept scrupulously clean,
2 good oestrus detection after service (by the livestock owner) should identify non-pregnant cows before any examination is carried out,
3 the operator of PD should never guess and should only give a diagnosis if he or she is completely satisfied that it is correct; it is better to re-examine a cow than to give a wrong verdict.

Pregnancy diagnosis is unreliable and slow when unskilled or semi-skilled persons attempt to do it. At the same time it is costly under such conditions. For these reasons it is usually more economical to hire a skilled and reputable person to do PD than to attempt to do it otherwise.
An additional advantage is (when a professional does the job) that veterinary service is available when it is needed.

As stated before (page 65), the ‘landmarks’ of greatest value in rectal examination are the cervix, the shaft of the ilium (hipbone) and the pubis (pelvic brim).

For quick and accurate genital examination, retraction (drawing back or in) of the open or early gravid (pregnant) uterus into the pelvic inlet is essential. Retraction is relatively easy in open (non-pregnant) cows and in cows which are not more than 90 days pregnant. Beyond 90 days it becomes more difficult.
Methods for retraction vary.
The following method is suggested as a guide to help students establish satisfactory procedures of their own.
For purposes of description left handed palpation is assumed.

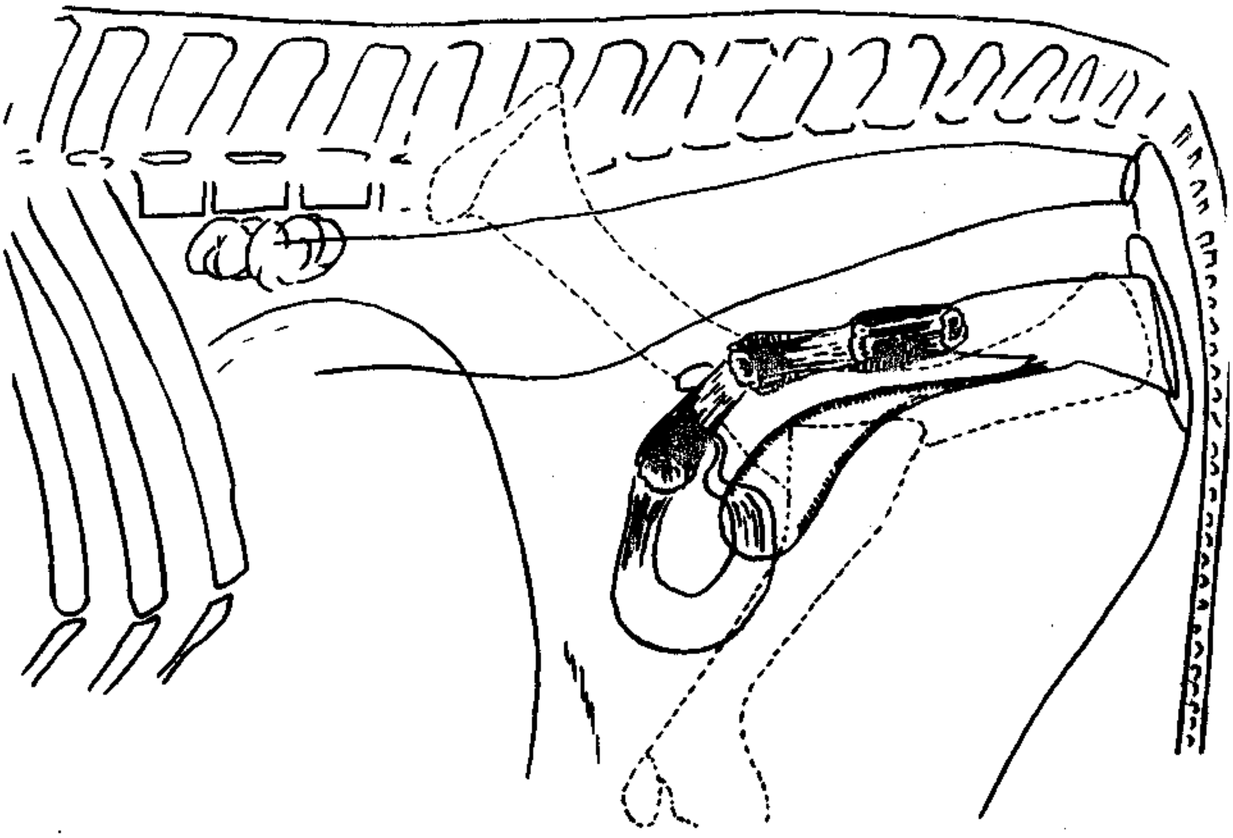

*Figure 20: Normal variations in the position of the cervix in the normal open cow*

The cervix may be found on the midline near the pelvic brim. It is a firm, cylindrical structure about eight cm long. It is located by sweeping the pelvic inlet with the edge of the open palpating hand. Do not use finger tips as they may damage the rectal mucosa. Normally the cervix will be found in this way.
If not, it is lying either over the brim of the pelvis or entirely within the pelvis. In that case repeat the procedure at various depths until the cervix is located.

Then elevate the cervix against the side of the pelvic inlet and insert the thumb **beneath** it. Rotate the hand so that the cervix is fixed between the ilium and the angle formed by the thumb and forefinger. At this time position the fingers dorsal to the cervix.
Flex the thumb and slide it forward along the cervix and the body of the uterus whilst keeping these organs slightly elevated and pressed between the thumb-forefinger angle and the pelvis. By sweeping the fingers laterally at this time, it is usually possible to grasp the left broad ligament. The broad ligament is felt as a band across the inside of the flexed fingers as the hand is retracted.

The thumb can now be placed in the angle of attachment of the broad ligament and the uterus; retraction can be maintained as the fingers sweep medially to grasp the left uterine horn. In some instances it is easy to cup the uterus in the hand at this time.
Retraction is completed by hooking a finger beneath the ventral intercornual ligament and tipping the genital tract back into the pelvis.

In some instances the intercornual ligament can be grasped by going directly to it and hooking it with the fingers. However, this technique is usually not successful.

In some pregnant cows the hand can be inserted over the brim of the pelvis and down beneath the uterus which is then cupped in an angle formed by the arm and the hand. It can then be retracted by gently moving it up and over the brim of the pelvis. Or, possibly, the membranes may be able to be slipped without retraction.

### Actual pregnancy diagnosis

If possible, the two uterine horns should be palpated throughout their entire length to determine their consistency. If the horns are empty they will have a meat-like consistency. If there is a pregnancy, fluids will be present in one or both horns.

A common mistake made by beginners is that of attempting to slip foetal membranes without determining the uterine contents. Sometimes a false slip is obtained and an open cow is called pregnant.

Four criteria can be applied to declare a cow pregnant:
1 palpation of the amniotic fluid
2 slip of foetal membranes
3 palpation of the foetus
4 palpation of placentomes (caruncles)

In addition, in advanced pregnancy the size and fremitus (which is the buzzing sensation resulting from turbulence produced by hypertrophy of the artery) of middle uterine arteries also give positive evidence of pregnancy.

Palpation of the *amniotic fluid* is possible from about day-30 to day-70 of gestation.
Before day-30 the amniotic vesicle is too small and after day-70 it becomes too large and too soft for palpation. Palpation of the amnion should not be done as a routine to determine pregnancy because of the risk of rupturing either the amniotic vesicle or the foetal heart sac; rupture will result in abortion. After 50 days the risk increases.

The *chorio-allantoic membranes* can be slipped in the interplacental areas because there are no normal uterine-foetal attachments in these areas.

Gently grasp a portion of the fluid-filled part of the uterine wall (in early pregnancy, the entire cross section of a horn) between thumb and forefinger and then release it gradually. The chorio-allantois and the uterine wall will slip between the thumb and forefinger in turn and this gives a typical 'slipping' sensation if the cow is pregnant.

As pregnancy advances the membranes fill progressively both uterine horns and the gravid horn becomes quite turgid. At ten weeks it is often easier to detect pregnancy by slip of membranes in the non-gravid horn because it is less tense.

The size and position of the uterus may be helpful indicators and may suggest pregnancy, but one should not call a cow pregnant unless a **positive sign of pregnancy is detected.**

Small *placentomes* can be palpated from 75 to 80 days. They become a more important diagnostic criterion in advanced pregnancy. Then relatively the amount of fluid has decreased and this makes them more evident.
Beginners who fail to orient themselves in the genital tract frequently make the error of palpating a caruncle, calling it an ova, and declaring a cow open when in fact she is in advanced pregnancy.

The *foetus* cannot be palpated until the amnion has become flaccid enough to allow manipulation. This is usually the case at about 75 days.

From then onwards palpation of the foetus is valuable for diagnosis and for estimating the duration of pregnancy.
In many cows the foetus is out of reach between about five and seven months.

*Hypertrophy of the middle uterine artery* can be palpated on the gravid side as early as 85 to 90 days after conception.
This structure is felt as a freely movable vessel which lies in the region of the shaft of the ilium. Make sure to distinguish it from the external iliac artery which is attached to the ilium.
The middle uterine artery can be felt as a cord four to five mm thick on the gravid side in early pregnancy; it progressively enlarges thereafter.
Fremitus may be distinguished as early as 90 to 120 days after conception, by holding the artery loosely between the fingers.
Too much digital pressure can produce a false fremitus.

**Ovaries**
The *ovaries* are located just lateral and caudal to the tips of the horns. They are easily located by tracing the horn with the hand until the tip is reached and then grasping outwards and slightly caudally.

As an alternative one can attempt to follow the mesovarium to the ovary, but this is more difficult.

The ovaries are flattened, almond-shaped organs approximately 3 × 2 × 1 cm. This shape can be distorted by the following functional structures:

1 The *follicle* is palpated as a smooth, rounded structure 1 to 2½ cm in diameter, which blends smoothly with the ovarian tissue. The texture varies with the stage of development.
  Smaller, early follicles are deeply embedded in ovarian stroma and feel quite hard.
  With advancing maturity, follicles may become fluctuating and blister-like.
2 The *corpus haemorrhagicum* is the structure which first develops after ovulation and it lasts until day-6 (5 days post ovulation).
  Following ovulation the ovulation depression (OVD) fills with blood.
  Surrounding luteal cells proliferate and hypertrophy to form the corpus haemorrhagicum which develops into the corpus luteum.
  In its early stages of growth the corpus haemorrhagicum is difficult to palpate. Later it is a soft structure with a smaller, softer crown than a developed corpus luteum.
3 The *corpus luteum (CL)* is palpable at about five days post ovulation. It frequently has a distinct crown about ½ cm in diameter and about ½ cm high; this crown projects from the surface of the ovary. The corpus luteum enlarges progressively to two to three cm by day-8 or 9 and has a liver-like consistency.
  After this, when there is no pregnancy, it regresses rapidly and becomes firmer and smaller.
  At the time of heat it can usually be palpated as a small, hard structure on one of the ovaries.
  The corpus luteum of pregnancy, however, becomes smooth, rounded and covered with epithelial cells after a month or two.

# 14 A brief description of the bovine embryotransfer technique

### Principle of embryo transfer

Embryo transfer (ET) is used to obtain *more calves* from *genetically highly valuable cows.*
These so-called donor cows are stimulated with hormones to release several eggs at one time ('superovulation'); normally only one egg is released at ovulation.
Some days after fertilization the embryos are flushed out and transferred into recipient cows.
The oestrus cycle of the recipient cow should be synchronus with the oestrus cycle of the donor cow; this can be achieved by applying oestrus-synchronizing treatments.
Collection of embryos can be repeated quite successfully on the same cow.

With a diagram:

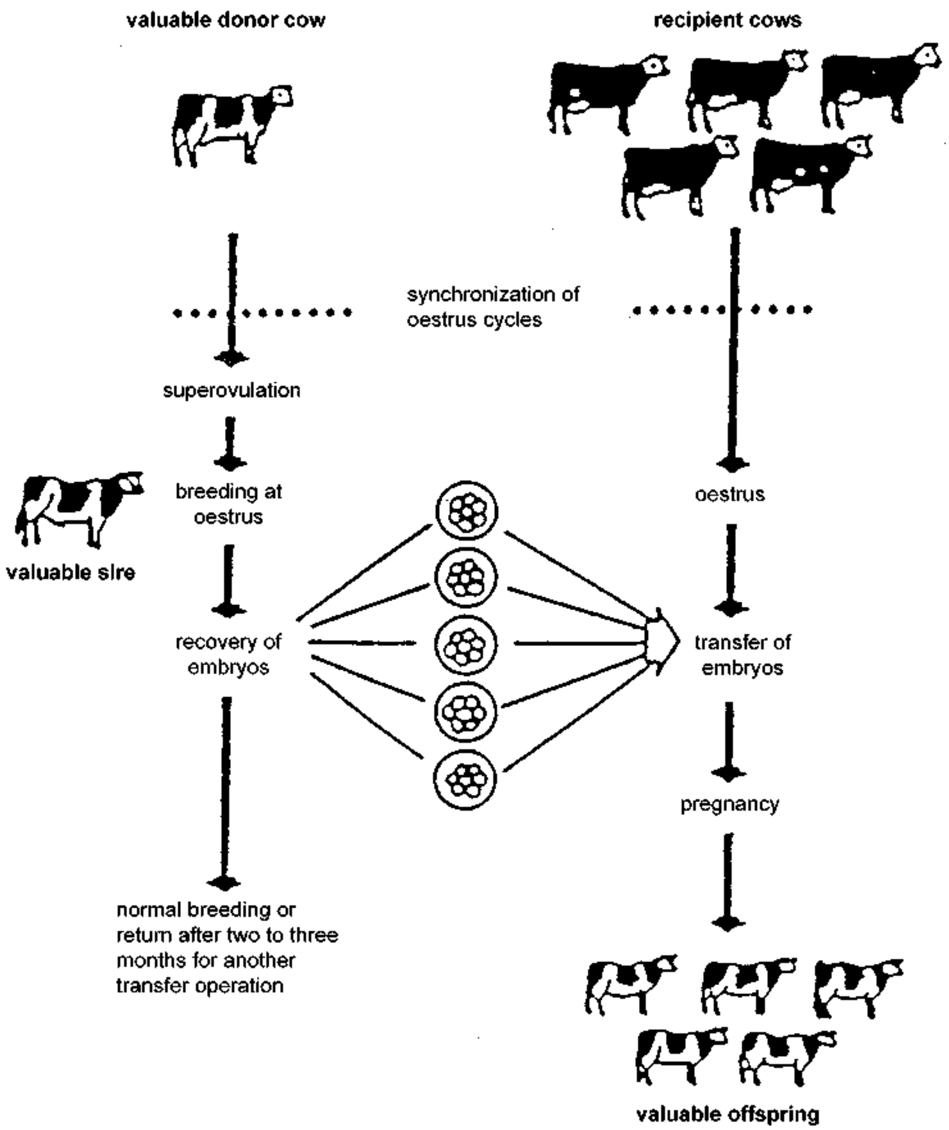


*Figure 21:*

### Superovulation

Superovulation can be induced by injecting FSH (follicle-stimulating hormone). FSH will stimulate follicles on the ovaries to mature.
Normally FSH from swine or sheep, or PMSG (pregnant mare serum gonadotrophin) is used.

Because of its relatively long-lasting effect, one PMSG injection is sufficient.
On the other hand, FSH has to be injected twice daily for 4 to 5 days (more effort is required).
A frequently used dosage is 3000 IU ('international units') PMSG or 2 × 7.5, 5.0, 5.0, 2.5 (2.5) mg FSH on four (or five) consecutive days.
The treatment is started between days-9 and 14 of the cow's cycle (day-0 = day of oestrus).

About 48 hours after the PMSG injection (or after the first FSH injection), a normal dosage of prostaglandin $F_{2\alpha}$ (or an analogue) is given in order to induce oestrus in the donor animal.
If PMSG is used, anti-PMSG has to be given in order to stop the long-lasting activity of PMSG.

The donor cow is usually served about 12 hours after the start of the standing heat period. Service has to be repeated 12 hours later if oestrus has not stopped by then.
The cycle of the recipient cows *must be synchronous with* that of the donor cow, in order to obtain good results (this is of the utmost importance!).
In most cases this means synchronizing recipient cows artificially. This can be done with one injection of prostaglandin $F_{2\alpha}$ about 12 hours before the donor cow is to be injected for oestrus induction.
It is also possible to synchronize the cycles with an artificial corpus luteum (a progesterone-releasing intravaginal device or an ear implant) for about ten days.

### Embryo collection

Embryos are collected non-surgically, **after** day-5 (arrival of embryo in uterine horn) and before day-8 (hatching from the zona pellucida).

Just after hatching the embryo is difficult to find, but between days-11 and 14 embryo collection is again possible.

In the first case embryos can be preserved for a long period by deepfreezing. And they can also be split into halves, triplets or quadruplets. Embryos collected between days-11 and 14 cannot successfully undergo these treatments.

But between days-11 and 14 it is very easy to find the embryos because of their relatively large size and this is a considerable advantage.
Before hatching the size of an embryo is about 0.15 μm, at day-12 about 5 μm and at day-14 from 50 to 100 μm (1 μm = 1/1000 mm).

Before starting the collection of embryos, the donor cow is checked for the number of corpora lutea and/or follicles. The rectum is emptied and a local aneasthetic is injected around the spinal cord on the tail head. This prevents strain during the flushing of the uterine horns.
Finally the vulval area is cleaned.

Then the embryos are collected by flushing the uterine horns one by one, with a Foley three-channel catheter held in position by the inflated collar at the tip of the catheter.
The catheter is connected with a tubing system or with a syringe filled to 100 ml.

The uterine horn is flushed several times with 25 to 100 ml fluid each time, depending on the size of the uterus.
The fluid used for flushing is usually a phosphate-buffered saline solution according to the formula of Dulbecco (PBS), supplemented with 1% heat-treated foetal calf serum (56 °C, for 30 minutes).
When both horns have been flushed, the collected fluid is examined for the presence of embryos. This is done by filtering the fluid through a filter system (φ 0.05 mm). The last 20 ml (with the embryos!) is flushed from the filter into a petri dish.

It is also possible to let the fluid collected from the donor cow stand for 30 minutes at least. This allows the embryos to settle and they can then be sucked up from the bottom by means of a 25 ml pipette; they can also be collected by removing the fluid that stands above the embryos.

The (remaining) fluid with the embryos is placed in a petri dish (φ about 10 cm).
This fluid is then examined with a dissecting microscope (magnification 20 x).

The embryos which one finds in the fluid must be checked for quality (magnification 50 x).
A normal embryo is in the right development stage and does not show signs of cellular degeneration (no loose cells, no dark spots).

Embryos which are found to be transferable are washed several times and then stored in fresh PBS, with 10-20% heat-treated serum.

### Embryo transfer
Embryos are sucked into a semen straw or into a special embryo transfer straw, depending on the transfer method that is applied (one embryo per straw).

The semen straws are used with an enlarged AI gun (length 50 cm), together with special, enlarged sheaths.
The embryo transfer straws have to be used with a special transfer apparatus.

With both methods, after the transfer gun has been loaded, a small plastic bag is placed around the transfer gun in order to prevent contamination in the vagina of the recipient cow.
When the tip of the transfer gun has reached the cervix (check by rectal palpation), the plastic bag is ruptured and the tip of the (still sterile) transfer gun is passed through the cervix into the uterine horn at the side of the corpus luteum containing ovary.
The embryo is placed as deeply as possible into the uterus, without damage to the uterine lining.

### Preservation of embryos
When there are more fresh embryos than recipient cows, the embryos can be preserved for later use.
Embryos can be preserved by cooling them down to temperatures lower than minus 130 °C.
Only good quality embryos should be deepfrozen; the freezing of low-quality embryos leads to poor results later on.

A cryoprotectant must be added before the freezing process is started; usually 1.4 M glycerol in PBS with 20% serum is added at 20 °C, in three steps.

The embryos are packed in straws or ampoules (plastic or glass) and are placed in a freezing chamber at minus 6 °C. Ten minutes later the cristallization is induced by touching the ampoules or straws with a very cold forceps; the medium freezes where they are touched (‘seeding’). Then the cristallization spreads throughout the ampoule or straw.
When the cristallization is complete, the cooling must be resumed until the temperature reaches minus 35 °C. The cooling rate must be about 0.3 °C/minute.

The material is stored in liquid nitrogen at minus 196 °C.

When the embryos are needed, they must be thawed in a water bath of 35 °C. The glycerol has to be removed; this can be done by bringing the embryos in 4 to 6 steps into a phosphate-buffered saline solution (+ 20% serum), each time with a lower glycerol concentration.
However, instead of using different glycerol concentrations to remove the glycerol, one can also use one sucrose solution (about 0.8 M). In fact sucrose solutions are used more and more.

### Splitting

To increase the number of calves per donor cow it is possible to split the embryos into halves, triplets or quadruplets.

The easiest way is to cut a morula (this is a 6 to 7 days old embryo) into two halves through the zona pellucida. Each half can lead to a normal pregnancy, with a normal pregnancy rate. So there will be twice as many pregnancies.
Some identical twins will be produced.

### Results

After superovulation one finds on average six normal, transferable embryos. With an average pregnancy rate of 55% this results in about three calves. With frozen (excellent quality) embryos a pregnancy rate of about 40% only can be expected.
With splitting embryos into halves one may expect a pregnancy rate of 110% (for instance, 10 non-cut embryos give 11 calves).
Splitting embryos into more than two parts will lower the pregnancy rate.

If embryo collection is done in a correct way, the donor cow is not damaged at all.
It is possible to repeat the process several times, at intervals of about two months.
To prevent pregnancy of the donor cow, her uterine horns are flushed with an embryo-toxic agent or the donor cow is given prostaglandin $F_{2\alpha}$.

The cost of embryo transfer is relatively high. In the Netherlands an ET calf costs about 200 US dollars as far as treatments are concerned. This means that the donor cows must be very valuable indeed!
In the Netherlands all treatments involved are carried out on the farm, by personnel employed by AI organisations.

The whole process of embryo transfer requires *specialized veterinary knowledge and experience*, as well as an *advanced breeding infrastructure.*

For many countries ET will as yet prove to be *too difficult and too costly* to implement on a regular basis.
The importation of frozen embryos, for instance to establish nucleus herds, seems more appropriate but whether it will justify the costs involved has to be studied in each individual case.

### Concluding remarks

The ideal recipient cow is a disease-free cow or heifer, cycling regularly, which can be of any breed.
The recipient cow should be in a well-nourished condition and be able to give birth to a calf the size of the implanted breed.

Successful embryo transfer activities require a *highly motivated and experienced staff* and *high capital investment* for facilities, equipment, drugs and experimental animals.

Possibilities of ET:
- ET can accelerate genetic progress in cattle breeding programmes,
- ET is an important research tool in studying genetic defects,
- ET can be used in research in general: reproduction physiology, fertilization, embryo implantation and pregnancy diagnosis,
- ET offers advantages over traditional transport of livestock between countries,
- the danger of disease transmission is reduced; for instance, BLV (bovine leukaemia virus), BTV (blue tongue virus) and FMDV (foot and mouth disease virus), provided that proper ET methods are used.

At present it is considered that AI still constitutes a more effective method of obtaining large-scale genetic gain. This is specifically true for those countries in which AI is still in its infancy and where milk recording and progeny-testing schemes are not yet established.

Bio-technology related to embryo transfer technology:

- in-vitro production of embryos (IVP)
- sex determination
- embryo splitting and cloning
- ‘genetic engineering’

*The text of this chapter has been prepared in cooperation with the late Dr H.Hoogenkamp*
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# Further reading

Relevant to dairy farming in warm climate zones, at farm level
AGROMISA Educational Materials:

- Basic calculations in agriculture and animal production.
  Instruction followed by exercises (with answers) at lower secondary level, for use in groups or for self-tuition. 80 pages, with illustrations in the text.
  Latest edition 1998.
- The farm as a commercial enterprise in a market economy.
  Elementary instruction followed by universally applicable exercises with answers, so that users gain a working knowledge of the subject. Latest revised edition 2003; 110 pages.
- Farm accounting. 50 pages, latest revised edition 2003.
- Modern dairy farming in warm climate zones (in three partner volumes). In total over 300 pages; latest version 2002.
- Tropical grasslands.
  Lecture notes made by the Department of Agronomy of the Wageningen Agricultural University and published by STOAS (latest version 1999).
- Video production on hand milking of dairy cows.
- Video production on embryo transfer in cattle.
- Foot care in cattle
  A guide complemented by a video production.