ARTIFICIAL INSEMINATION

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Introduction

Artificial insemination, or AI for short, is the introduction of spermatozoa into the female reproductive tract around the time of oestrus for the purpose of achieving conception. AI is one of the most powerful tools developed in the twentieth century for the genetic improvement of livestock. It enables the application of high selection pressure on the male side thereby substantially increasing the rate of genetic progress per year.

Advantages

The advantages of AI include the following:
- It enhances the rate of genetic progress in a herd or flock.
- It reduces cost of service in several situation compared with natural mating.
- It solves time and space constraints in animal breeding. This is as a result of ability to preserve semen for long periods.
- It reduces the risk of venereal disease transmission which is a real possibility with natural mating.
- It enables better planning of the breeding and production programme due to the maintenance of accurate breeding records.
- It promotes world trade in animal genetic resources

Disadvantages

AI has the following disadvantages:
- It requires infrastructure often unavailable in developing countries. These include good communications, steady power supply, industrial supply of cryogens, etc.
- Skilled technicians are in short supply in rural areas.
- AI sometimes comes into social conflict as typified by some nomadic herdsmen who reject AI progeny as "bastards".
- AI is not amenable to nomadic production system.
- Lack of clearly defined breeding may render AI ineffective.

Semen collection

The most common method of semen collection especially from bulls, rams and bucks is by artificial vagina (AV), using a teaser animal or dummy. An artificial vagina is a device consisting of a rubber hose in which a rubber lining

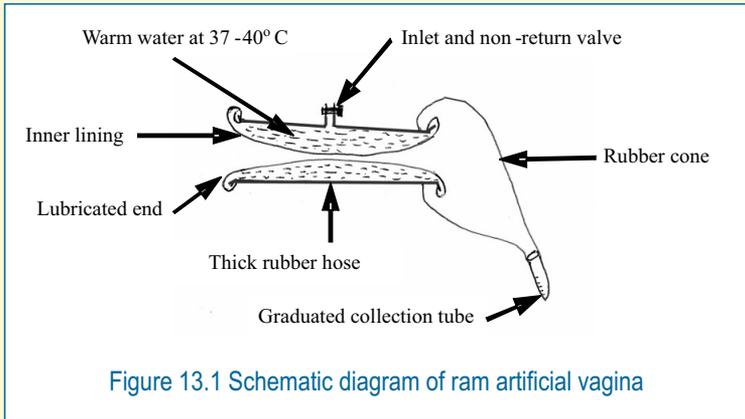is inserted to create an air-tight compartment usually filled with warm water through a valve (Fig 13.1).

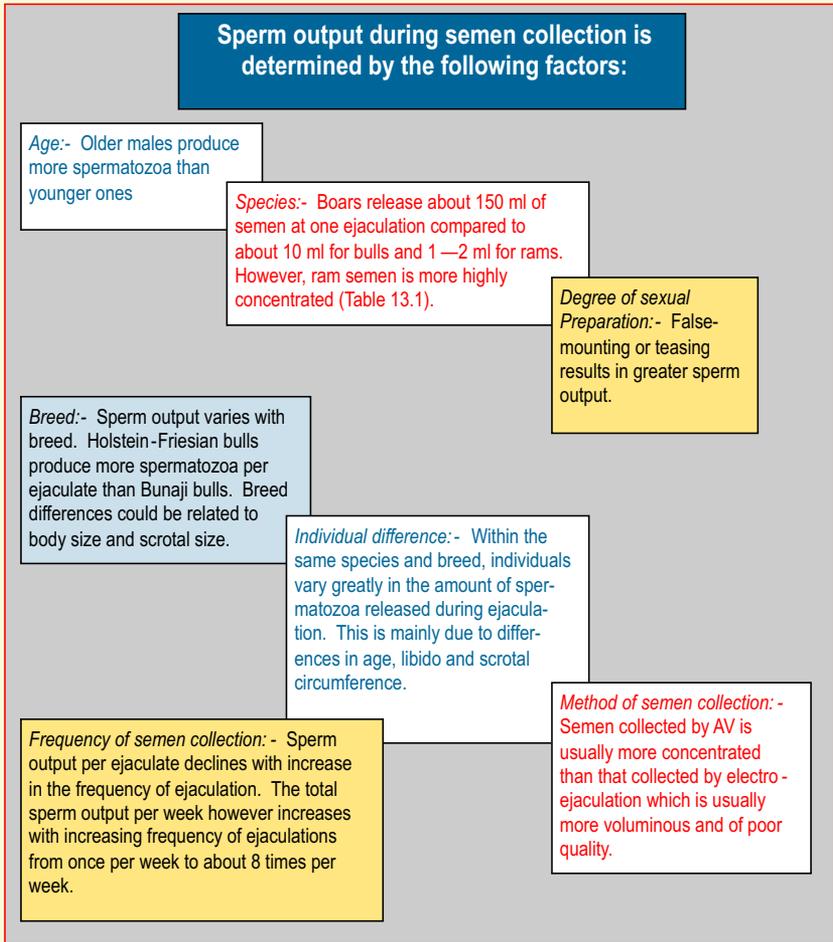Figure 13.1 Schematic diagram of ram artificial vagina

Air is blown into the water-filled compartment till the inner thorough surface of the AV closes. The end of the AV through which the penis will be inserted is lubricated with petroleum jelly while the other end is capped with a rubber cone at the end of which a test-tube is filled into which the ejaculated semen drains.

A *teaser* is a female (or sometimes male) animal restrained in a semen collection crate for mounting by the male from which semen is to be collected by AV. As the male animal is led to the teaser, it attempts to mount it but is pushed down at least twice in a procedure known as false mounting. This heightens the male's sexual urge and results in the ejaculation of higher quantity of semen. As the male mounts the teaser for the third time, the protruding penis is deflected into the AV and it ejaculates. A lounging movement by the male signals ejaculation which takes less than 30 seconds in the ram and the bull. The male dismounts and the AV is held upright to allow the semen to drain properly into the test tube.

Semen collection in the boar is by the gloved-hand technique. The technician wears a rubber glove on one hand with which the screw-like end of the boar penis is held firmly after mounting the dummy, to mimic the lockingin in the cervix. A flask holding three large test tubes (250 ml each) is used to receive the ejaculated semen in fractions. The boar semen is released in distinct fractions sequentially as pre-sperm, sperm rich and post-sperm portions. The pre-sperm fraction is a relatively clear sperm-poor fluid comprising of preputial secretion. The sperm-rich fraction is a creamy-white fluid with high concentrations of spermatozoa suspended mainly in vesicular gland secretions.

The post-sperm fraction is quite voluminous with few spermatozoa but plenty of gelatinous secretions from the bulbo-urethral glands. Ejaculation in the boar lasts from 10 to 30 minutes.

Semen collection is sometimes undertaken using an electro-ejaculator. This is an electrical probe inserted in the male's rectum used to deliver intermittent voltage surges from a battery pack for electrical stimulation of the nerves around the accessory sex glands, leading to involuntary ejaculation. The semen is usually more voluminous and of poorer quality than that collected by AV. The male animal has to be well restrained during electro-ejaculation to contain any violent reaction arising from the electric shocks.

Sperm output during semen collection is determined by the following factors:

Age:- Older males produce more spermatozoa than younger ones

Species:- Boars release about 150 ml of semen at one ejaculation compared to about 10 ml for bulls and 1—2 ml for rams. However, ram semen is more highly concentrated (Table 13.1).

Degree of sexual Preparation:- False-mounting or teasing results in greater sperm output.

Breed:- Sperm output varies with breed. Holstein-Friesian bulls produce more spermatozoa per ejaculate than Bunaji bulls. Breed differences could be related to body size and scrotal size.

Individual difference:- Within the same species and breed, individuals vary greatly in the amount of spermatozoa released during ejaculation. This is mainly due to differences in age, libido and scrotal circumference.

Method of semen collection: - Semen collected by AV is usually more concentrated than that collected by electro-ejaculation which is usually more voluminous and of poor quality.

Frequency of semen collection: - Sperm output per ejaculate declines with increase in the frequency of ejaculation. The total sperm output per week however increases with increasing frequency of ejaculations from once per week to about 8 times per week.

Semen Evaluation

Semen to be used for AI has to be evaluated after collection to ascertain its quality. The evaluation starts from the appearance. Good quality semen appears creamy white. Translucent semen indicates low concentration. Presence of blood stains or unusual colour signifies poor quality or contamination. The semen volume is usually determined from the graduation on the collection tube.

Sperm motility is determined under an ordinary light or phase-contrast microscope fitted with a warm stage at x 400 magnification by viewing a drop of semen on a glass slide under cover slip. Semen having sperm motility above 65% can be considered good. For more accurate estimates of percentage of motile spermatozoa and sperm velocity, time-lapse, photomicrography can be used.

Spermatozoa concentration can be determined by haemocytometer counts of diluted semen. It is more common however to take absorbance readings of diluted semen using a spectrophotometer at 600 nm calibrated against haemocytometer counts. The percentage of live spermatozoa can be determined from semen smears stained with eosin-nigrosine stain. Spermatozoa which were presumably alive at the time of staining appear whitish as they do not absorb eosin while dead spermatozoa pick up eosin stain and appear red.

Sperm morphology can be ascertained by phase contrast microscopy at x 1,000 magnification under oil-immersion. Good quality semen would have incidence of sperm abnormalities of below 5%. Occasionally, high levels of sperm abnormalities are encountered which signifies poor quality semen.

Table 13.1 Ejaculate characteristics of the bull, ram and boar

Trait	Bull	Ram	Boar
Volume, ml	3-10	0.3-1.2	150-300
Sperm concentration x 10^9/ml	0.6-1.2	1.5-4.0	0.2-0.4
Motility, %	60-80	60-95	50-80
Normal sperm, %	65-95	80-95	70-90
pH	6.9	6.9	7.5

SEMEN PRESERVATION

Principles

The storage or preservation of semen involves the slowing down of the spermatozoa's metabolic rate. This involves either the cooling down of the cells or chemical inhibition of its metabolism for instance by carbon dioxide gassing to create anaerobic conditions. Preservation also involves the elimination or control of injurious metabolic by-products through the use of an effective buffer, dilution or dialysis. It also involves the supply of an exogenous energy source such as glucose, the maintenance of cell integrity through the use of egg yolk or milk to prevent "cold shock" during cooling, or the use of cryoprotective agents such as glycerol or erythritol to reduce or prevent "freezing injury" during deep frozen storage. Preservation also involves the inhibition of autolysis.

Methods

There are two main methods of liquid storage of semen, namely ambient temperature storage and chilled storage at 5°C. A third method of liquid storage which is less common is flow dialysis. Continuous flow dialysis involves the continuous but slow replacement of the buffer in which a semen-filled dialysis bag is suspended. Discontinuous flow dialysis involves the complete replacement of the dialysis fluid once every 24 hours. Liquid storage of semen is for short periods only, lasting from a few hours to a few days. The spermatozoa lose viability with length of storage.

Long term preservation of semen is by deep frozen storage. Semen samples are diluted appropriately and cooled slowly to 5°C before freezing in pellets on dry ice (solid carbon dioxide) or in straws in liquid nitrogen vapour. The frozen semen is then stored in canisters of liquid nitrogen vapour. The frozen semen retains fertility for several years.

Semen extenders

Diluents or extenders are essentially buffers added to semen primarily to increase the volume to obtain more insemination doses for AI from the ejaculate. They also play a significant role in semen preservation. The composition of some diluents is shown in Table 13.2.

Table 13.2 Composition of some diluents

Components	Diluents		
	Tris-yolk	**Yolk-citrate**	**Milk-yolk**
Tris*, g	3.078	-	-
Citric acid, g	1.675	-	-
Tri-sodium citrate, g	-	2.9	-
Fructose, g	1.250	-	-
Milk**, ml	-	-	100
Egg yolk, ml	20	20	20
Sodium penicillin G, IU	100,000	100,000	100,000
Dihydrostreptomycin sulphate, g	0.1	0.1	0.1
Distilled water, ml	To 100	To 100	-

* 2-Amino-2-(hydroxymethyl) propane -1, 3-diol
** Cow or goat milk, heated to 92 ° C for 10 minutes

The properties of a good semen extender include the following:

(a) It must have a suitable osmotic pressure of between 290 mOsm and 320 mOsm.

(b) It should have a pH of 7.0 or slightly higher, as spermatozoa tolerate basic better than acidic conditions.

(c) It must provide adequate buffering against toxic end-products of cell metabolism.

(d) It should contain an energy source.

(e) It must contain antibiotics for bacterial control.

(f) It should contain substances capable of protecting the spermatozoa against temperature shock.

(g) It must be free of substances detrimental to fertilization, implantation and embryogenesis.

Factors affecting spermatozoa survival during preservation

The major factors which determine the survival of spermatozoa during storage include the following:

- Initial semen quality before storage
- Processing and storage method
- Diluents composition
- Length of storage

Insemination

Artificial insemination could be with fresh, diluted or preserved semen. Female animals to be inseminated should be on heat and should be isolated from the rest of the herd or flock. Insemination could be by means of a pipette or insemination gun. The females should be checked for heat twice daily, in the morning and afternoon. They should be inseminated 24 hours after oestrus detection.

Factors affecting fertility during AI include the following:

- Semen quality
- Timing of insemination in relation to oestrus
- Insemination dose
- Fertility of the female
- Technicians' skill.