COURSE GUIDE

ANP 514
TECHNIQUES IN ANIMAL REPRODUCTION
(2 UNITS)

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COURSE GUIDE

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INTRODUCTION

Techniques in Animal Reproduction, discusses some novel techniques in reproductive technology, such as artificial insemination, sex reversal, gamete and embryo sexing. The concepts of induction and synchronization of oestrus and ovulation are also discussed in some of the units. In some of the units, multiple ovulation and embryo transfer techniques are also discussed. Other topics discussed in other units include heat detection methods, cryopreservation techniques, pregnancy diagnosis, induction of parturition and lactation.

In some units, weaning methods, invitro-maturition and fertilization are discussed. Some two units form a module. Some of the more recent assisted reproductive techniques like DNA probing; nuclear transfer and cloning are discussed in the later modules and units. You will also come across the debate on ethical issues pertaining to some of these techniques like cloning.

Assignment File: The assignment file will be made available in this file. You will find all the details of the work you must submit to your tutor or facilitator for marks. The marks you obtain for these assignments will count towards the final mark you will obtain for this course. Assignments will normally attract 30% of the final grade, while the final examination attracts 70%.

Four assignments are picked to make up to over 30% marks of the assignment. As a precaution, you are advised to keep a copy of each assignment you submit if it is not computer based. You are advised to be very systematic in following the instruction as it pertains to your course of study.

Final Examination and Grading: The final examination of the course (ANP 514: Techniques in Animal Reproduction) will have five questions for you to answer any four in 2 hours. Total marks earned will be over 70%, while the tutor or computer marked assignments (TMA or CMA) will be over 30% as earlier stated. Examination questions can come from any part of the course.

You may find it useful to review all TMAs/CMAs and Students' Assessment Exercises before the final examination

Working Through this Course: For you to be will taught, you ought to read through all the study units, and some of the references and other materials. In each unit under each module, there are Students' Assessment Exercises which you should attempt before checking the correct answers at the end of the unit. TMAs/CMAs must be submitted for marking and grading before the final examination, to test your understanding and mastery of the course.

Tutor-Marked Assignments (TMAs) or Computer–Marked Assignments (CMAs): There are Tutor-Marked Assignments or Computer-Marked Assignments in this course. You are advised to do and submit four TMAs/CMAs. The completed TMAs /CMAs will be graded and the best 3 collated to form 30% of your final examination, which is 70%, both add up to 100%.

Below are some points worth noting:

1. Read the course guide thoroughly;
2. Organize a study schedule;
3. Do everything you can to stick to your study schedule.
4. Review the objectives of every unit and make sure you have achieved the objectives. Any further explanation can be received through your facilitator or in your students' study cycle.
5. After going through the units, review the course and prepare yourself for the final examination.

Final Advise: Studying in any Open and Distant Learning (ODL) mode involves self-study and discipline. Manage your time well. Take advantage of any facilitation or study cycle to ask any questions on units you do not understand. Proceed unit by unit through the course, pacing your studies as necessary so that the whole exercise will be easy for you.

Best of Luck.

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MODULE 1: SEX DETERMINATION, MANIPULATION AND HEAT DETECTION METHODS

Introduction

In this module, you have 2 units; the first unit covers sex determination while the second unit has to do with heat detection methods in farm animals.

The objective of the module is to enable you

i. understand sex determination and manipulation in farm animals
ii. Get to know heat detection methods in farm animals.

UNIT 1: SEX DETERMINATION AND MANIPULATION

2.0 Introduction

In this unit you will be studying about sex determination and manipulation in farm livestock, birds and some reptiles. In fact you will learn how to predetermine the sex of the offspring.

1.1 Objectives

At the end of this unit, you should be able to

- Explain clearly sex determination in mammals and most insects;
- List methods of sex predetermination;
- Explain the concept of sex reversal.

1.2 Sex

Sex is defined as the sum total of those differences in structure and function on the basis of which an organism is classified as male or female.

1.3 Sex Determination

Two main theories have been advanced in explaining the mechanism of sex determination.

1.3.1 Chromosomal Theory of Sex Determination

Under this theory, sex is said to be determined at fertilization by the sex chromosomes. Each gamete contains the haploid number of chromosomes. In mammals and in most insects, all gametes produced by the female are similar having X chromosomes. Males on the other hand produce two types of gametes in approximately equal numbers, one type bearing the X chromosome and the other type bearing the Y chromosome.

In mammals and most insects, the female is known as the homogametic (xx) sex while the male is heterogametic (xy). At fertilization, a zygote with XX results is a female, while that with XY results is a male. In birds, the female is the heterogametic sex while the male is homogametic. These are illustrated in table 1 below;

Table 1: Kinds of Chromosomal Sex Determination

ORGANISM	HETEROGAMETIC	GAMETES	ZYGOTES

	SEX	SPERM	EGGS	FEMALE	MALE
Mammals and most insects	Male	X and Y	All X	XX	XY
Grass hoppers	Male	X and O	All X	XX	X
Birds, some reptiles and amphibians	Female	AIIZ	Z and W	ZW	ZZ

Adopted and Modified from Osinowo, 2006, Introduction to Animal Reproduction.

Aberrations of genetic sex do occur either as a result of non-disjunction of sex chromosomes translocation, deletion or mutation. In maternal non-disjunction, fertilization of the ovum will lead to formation of an XXX or an XXY zygote. In paternal non-disjunction, zygotes of XXY or XO genotypes result. Such cases of aneuplody often result in gonadal or endocrine defects.

In man, the Klinefelter's syndrome (XXY) is characterized by gonadal hypofunction. In women, Turner's syndrome (XO) is characterized by gonadal agenesis or aplasia.

1.3.2 Genic Balances Theory of Sex Determination

This theory purports that sex is a phenotypic trait, determined by interaction between genotype and environment. Individuals vary in their degree of maleness or femaleness.

C.B. Bridges in 1922 proposed the genic balance theory to explain apparent quantitative variability in sexual character. According to this theory, sex is determined by the autosomes as well as by the X chromosomes, the ratio of autosomes to X's being the significant relation. In Drosophila, the X chromosome carries more genes for maleness (Table 2). Which sex actually develops is decided by the balance between the two sets of genes.

Table 2: Sexual Types in the Fruit Fly, *Drosophila melanogaster*

SEX	X CHROMOSOME	SETS OF AUTOSOMES (A)	SEX INDEX (X/A)
Super female	3	2	1.5
Normal Female; tetraploid	4	4	1.0
triploid	3	3	1.0
diploid	2	2	1.0
haploid	1	1	1.0
Intersex	2	3	0.67
Normal male	1	2	0.50
Super male	1	3	0.33

Adopted from O.A. Osinowo, 2006, Introduction to Animal Reproduction

1.4 Other Theories of Sex Determination Include:

1.4.1 Genetic Dosage Compensation X – Inactivation Model of Sex Determination

The ZFY gene, thought to constitute the primary sex-determining signal, was identified within a very small segment of the Y chromosome by Page and co-workers in 1987.

However, the presence of a similar gene, ZFX, on the X chromosome prompted the propounding of the dosage compensation X-inactivation theory of sex determination.

According to this theory, both ZFX and ZFY produced functionally interchangeable proteins. It therefore means that XY cells would have two active copies of the gene (ZFX and ZFY) while XX cells would only have one active (ZFX) copy, due to X-inactivation. Embryos with two copies would thus develop into males while those with a single copy of the gene would develop into females.

In this way, the theory held that gene dosage determined sex. Though elegant, the theory became untenable when it later became evident that the ZFX gene escapes X-inactivation, thus contradicting the dosage compensation model.

1.4.2 Sex Determination in Birds

In order to distinguish animals with female heterogamety from those with male heterogamety, the distinguishing chromosome is known as W in the former (in mammals, Y), while the common chromosome is called Z (X in mammals).

1.4.3 Temperature Dependent Sex Determination in Alligators

Many reptiles, including alligators and crocodiles, exhibit no sex chromosome dimorphism. Rather, the sex of the offspring is determined by the temperature at which the eggs are incubated.

In alligators (Alligator mississippiensi), incubation of eggs from:

29-31.5°C - results in 100% female offspring
32.5-33°C - results in 100% male offspring
32°C
33.5-34.5°C - different sex ratios
35°c – 100% females
Below 29°C and above 35°C, high mortality results.

1.4.4 Sex Determination in Fishes

Most fishes function either as male or female throughout life, i.e. most are bisexual or gonochoristic, as opposed to hermaphroditic, a condition in which an individual produces both eggs and sperm at some stage of its development. Establishment of sex depends on sex chromosomes, designated X and Y in most fishes.

> Usually, XX individuals are female
> and XY individuals are male

There are exceptions, e.g. among the poeciliidea in which the homogametic individual is male. Chromosome designated W and Z are recognized in some of these fishes. Although the sexes have similar appearances in many species, sexual dimorphism or dichromatism is common in fishes and may be especially well marked in those species with internal fertilization or elaborate reproductive behavior. Occasional hermaphrodites are found in many gonochoristic species as an abnormality, but numerous species are normally hermaphroditic, with some even capable of self-fertilization.

Synchronous (or simultaneous) hermaphrodites have ripe ovaries and testes at the same time but usually spawn with one or more other individuals, alternately taking the role of male and female. Synchronous hermaphrodites are known from the following families: Chlorophthamidae, Bathypteroidae, Alepisauridae, etc. Protandrous condition is known in members of Gonostonatidae, Serranidae, Sparidae, etc. Protogynous hermaphrodites have been noted in the Synbranchidae, Serranidae, Meanidae and Labridae.

Sex reversal and hermaphroditism are controlled by the endocrine system which is genetically programmed in normally hermaphroditic species to act on the gonads in response to the proper stimuli, which can be internal or external.

1.5 Pre-Determination of Sex

X and Y chromosome bearing sperm have differences in quality of DNA. The X having more DNA than Y chromosome-bearing sperm.

a. Bull, boar and ram X and Y chromosome-bearing sperm population can be separated by flow-cytometric procedures.
b. Measurements of DNA in separated X and Y-bearing sperm populations can be used to predetermine the sex of offspring.
c. Centrifuging of semen results in the Y-chromosome bearing sperms being in the upper half of the supernatant, while the X-chromosome bearing sperm are in the remaining half supernatant below.
d. In humans semen deposition into the female reproductive tract at peak body temperature or the sudden fall in temperature in normal female cycles results in a male zygote. Ovulation is said to take place at either peak body temperature or the sudden fall just after the peak. Y-chromosome bearing sperm are said to be lighter than X and therefore swim and reach the egg before the X, but the Y are less viable than the X.

1.6 Sex Reversal, Gamete and Embryo Sexing

Sex reversal is common in some species of fishes as earlier discussed. See 2.4.4
With the development of the embryo transfer technology, interest in the sexing of gametes before AI or embryos before transplantation has become substantial. Gamete sexing differentiating the Y-chromosome bearing sperm has been earlier discussed. Possible cytological approaches in sexing of embryos before transplantation are:

a. Biopsy of bovine embryos on day 6 or 7
b. Observation of the Barr body
c. Trophoblast biopsy of bovine embryos between days 1 and 15, and
d. Halved embryos on days 6 and 7

Of these four methods, only trophoblast biopsy on days 12-15 and embryo bisection on days 6 or 7 can be considered practicable. The first excludes the possibility of long term storage while the second requires additional expense and expertise.
Embryo sexing using immunological techniques has also been reported. Experimental evidence suggests that H-Y antigen is present in early embryos of the bovine, and that serological reagents that identify H-Y antigen can be used to identify males and females in embryo transfer systems.

Student Self-Assessment Exercise (SAE) 1.1
List Any Three (3) Methods of Pre-Determining Sex

1.7 The Sex Chromatin

The sex chromatin is a well-developed DNA-positive chromocentre, observed in the nucleus of most cells from certain tissues in most mammals. It represents a heterochromatic X chromosome which is genetically inactive. It usually lies against the inner surface of the nuclear membrane or adjacent to the nucleolus. The maximum number of sex chromatin bodies in a given interphase nucleus is one less than the number of X chromosome. Thus, XY or XO individuals are sex-chromatin negative, while XX or XXY have one sex chromatin body per nucleus.

In farm animals, sexual dimorphism as indicated by presence of the sex chromatin is detected mainly in neurons of the brain and spinal cord. The sex chromatin is also detectable in foetal cells from the amniotic fluid of cattle, sheep and pigs.

1.8 Conclusion

This unit attempts to give a brief overview of sex determination and manipulation in animals. The sex chromosomes and which of the sexes are the sex determinants have also been outlined, as well as some processes of sex pre-determination.

Answer to SAE 1.1

Three methods of predetermination of sex will include

1. Flow – Cytometic procedure
2. Measurement of DNA in separated X and Y – bearing sperm population.
3. Centrifuging of semen.

1.9 Summary

Sex determination and manipulation in farm animals is a complex technique, some require specialized equipment. While in some species the males are heterozygous, in others the females are heterozygous.

1.10 Tutor-Marked Assignment

How is sex determined in fishes?

Module 1 (Unit 1)

Question:

How is sex determined in fishes?

Answer:

Most fishes function either as male or female throughout life. i.e. most are bisexual or gonochonistic, as opposed to hermaphroditic, a condition In which an individual produces both eggs and sperm at some stage of its development. Establishment of sex depends on sex chromosomes, designated X and Y in most fishes.

Usually, XX individuals are female and XY individuals are male. There are exceptions, e.g. among the poeciliidea in which the homogametic individual is male. Chromosome designated W and Z are recognized in some of these fishes. Although the sexes have similar appearances in many species, sexual dimorphism or dichromatism is common in fishes and may be especially well marked in those species with internal fertilization or elaborate reproductive behaviour. Occasional hermaphrodites are found in many

gonochoristic species as an abnormality, but numerous species are normally hermaphrodic, with some even capable of self – fertilization.

1.11 References And Other Resources

Osinowo, 2006, Introduction to Animal Reproduction, Sophie Academy, UNAAB, Abeokuta, Nigeria.

Sorensen Jr. A.M. Repro Lab.A Laboratory Manual for Animal Reproduction.3rd EditionKendall/Hunt Publishing Company, U.S.A.

UNIT 2 HEAT DETECTION METHODS

2.0 Introduction

In this unit, you will study the various methods of heat detection in farm animals. What is also done in practice will be shown.

2.1 Objectives

At the end of your studying this unit, you should be able to:

- Properly identify signs of heat in some farm animals;
- Know what to do in practice in some farm animals in heat detection.

2.2 Heat Detection Methods

The heat (oestrus) period is the time within the oestrous cycle when the female animal stands to be mounted and mated by the male. The oestrous cycle refers to the cycle from one period of heat to the other in the same female animal.

Heat (oestrus) detection becomes necessary when:

1. Animals are to be artificially inseminated.
2. There is need to keep accurate breeding records
3. Checking for absence of pregnancy after breeding.

Males have an inherent capability and are best detectors of females on heat. However, there are characteristic signs of oestrus for each species.

2.3 Signs of Oestrusin Some Farm Animals

Table 3: Signs of Oestrus

Species	Observable signs				
	Swollen vulva	Mucous secretion	Behavior	Absence of male	Presence of male
Sow	Yes	No	Restless, irritable, explorative, frequent urinations, vocal emissions	Immobility response to man	Stands to man
Ewe	No	NO	None	None	Stands to male
Cow	Sometimes	Sometimes	Restless, nervous, off feed herd mates	Stands for herd mates	Stands for either male or herd mates

Source: E.S.E. Hafez, 3rd edition. Reproduction in Farm Animals.Pg. 432.

2.4 Heat Detection in Practice

1. In sows, intact boar can be introduced into a pen of females and observation made of those females the boar attempts to mount. Back pressure can also be applied on the suspected sow on heat and she could be immobile if on heat.
2. In ewes, vasectomized rams can be fitted with marking harnesses containing marking crayons. The raddled ram can be made to run with the ewe flock. Ewes on heat will stand to be mounted and marked.
3. In the cow, intact or vasectomized bull can be fitted with a chin-ball marker and introduced into a herd of cows. Cows on heat will stand to be mounted and marked.

Students' Assessment Exercise (SAE) 2.1
Question: Who is the best detector of heat?

2.5 Conclusion

Heat detection is very important in livestock management especially where females are kept separate from the males. Knowing the signs of heat is therefore important for optimum reproduction.

Answers to Students' Assessment Exercise 2.1
The best detector of heat is the male animal.

2.6 Summary

In this unit, you have studied the various signs of oestrus in some farm animals and what obtains in practice as far as heat detection is concerned. This should enhance reproduction management especially where females are kept separate from the males.

2.7 Tutor-Marked Assignment

Write notes on heat detection in some farm animals in practice.

2.8 Reference And Other Materials

King J.O.L. 1978.An Introduction to Animal Husbandary. Blackwell Scientific Publishers,Britain

Osinowo O.A. 1986. Reproductive Physiology Manual, NAPRI, ABU Zaria.

MODULE 2: CRYOPRESERVATION AND ARTIFICIAL INSEMINATION

1.0 Introduction

In this module you will have 2 units, the first unit deals with cryopreservation techniques, while the second unit handles the issues of artificial insemination (AI).

The objectives include

i. To enable you understand the cryopreservation techniques available

ii. To enable you know the advantages and disadvantages of AI and how it is done.

UNIT 3: CRYOPRESERVATION TECHNIQUES

3.0 Introduction

This unit will treat in general the concept of cryopreservation, the steps and techniques as a reproductive tool in farm animal management.

3.1 Objectives

By the time you have studied this unit, you should be able to:

- Define cryopreservation;
- Give some examples of samples that can be cryopreserved;
- List the steps in cryopreservation of cells

3.2 Cryopreservation Technique

Cryopreservation is a process where cells or whole tissues are preserved by cooling to sub-zero temperature, typically 77K (= -196°C, the boiling point of liquid nitrogen). At these cold temperatures, all biological activity, including the biochemical reactions that would cause cell death, is effectively stopped. However, ifcryoprotectant solutions are not used, the cells being preserved are likely to be damaged due to freezing during the cooling or thawing process.

Generally, cryopreservation is easier for thin samples and small clumps of individual cells, because these can be cooled more quickly and so require lesser doses of toxic cryoprotectants. Therefore, cryopreservation of human livers and hearts for storage and transplant is still impractical.

Nevertheless, suitable combinations of cryoprotectants and regimes of cooling and rinsing during warming often allow the successful cryopreservation of biological materials, particularly cell suspensions or thin tissue samples. Examples include:

- Semen in semen cryopreservation
- Blood
    - Special cells for transfusion
    - Stem cells. It is optimal in high concentration of synthetic serum, step wise equilibration and slow cooling.
    - Umbilical cord blood
- Tissue samples like tumors and histological cross sections.
- Eggs (oocytes) in oocyte cryopreservation
- Embryos that are 2,4,or 8 cells when frozen in embryo cryopreservation
- Ovarian tissue in ovarian tissue cryopreservation
- Plant seeds or shoots may be cryopreserved for conservation purpose

Additionally, efforts are underway to preserve humans cryogenically, known as cryonics. For such efforts either the brain within the head or the entire body may experience the above process. Cryonics is in a different category from the afore mentioned examples, however, whole countless cryopreserved cells, vaccines, tissues and other biological samples have been thawed and used successfully, this has not yet been the case at all for cryopreserved brains or bodies.

Students' Assessment Exercise 3.1

Question: Which cells will you recommend for cryopreservation as far as reproduction is concerned?

3.3 Cryopreservation of Cells – Steps And Techniques

1. Cultures to be cryopreserved should be healthy, free from contamination, and should be maintained in log phase growth for several days before freezing.

2. Grow attaching cell culture to late log phase, trypsinize and centrifuge. If freezing suspension cells, only centrifugation is necessary.

3. Resuspend cells in sterile serum-containing culture medium containing 10% v/v dimethylsulfoxide (DMSO). Work should proceed quickly to minimize the length of time the cells are exposed to DMSO in the liquid state. The highest purity DMSO should be used and preferably, it should come from a bottle that has not been previously opened or exposed to light for long periods of time.

4. Place the appropriate volume and cell number into cryopreservation ampules – usually 2×10^6 cells/1ml ampule. Plastic or glass ampules may be used. However, plastic ampoules with external silicone seals function best when kept above liquid nitrogen temperature (i.e. in the vapor phase). Immersion into the liquid nitrogen phase can result in liquid nitrogen entering the ampule spraying out during the defrosting procedure. If storage in liquid nitrogen is preferred, plastic ampules with internal "O" rings perform satisfactorily.
   Glass ampoules offer the best result due to the secure seal and the rapidity with which the ampule can be defrosted, thereby allowing for higher culture viability. However, they can be inconvenient to use due to the requirement of flame sealing.

5. Place the ampules in a controlled-rate freezer and cool at a rate of 1°c/minute. If a controlled-rate freezing apparatus is not available, adequate results can be obtained by:
   a. Placing the ampule inside a 1-inch foam-insulated box and keeping the box at –70°C for 12 hours.
   b. Cooling the ampules in the liquid nitrogen phase using a liquid nitrogen canister insert.
   c. Placing the ampules in an isopropanol bath that is subsequently cooled in a -70°C freezing.
   d. Placing the ampoules directly into a -20°c freezer for several hours and then transferring to a -70°C for further cooling.
   e. Placing the ampules directly into a -70°C freezer.

      The last two methods (d and e) are not ideal since the culture viability can be affected and result in the loss of sensitive populations. These methods should only be used when no other options are available.

6. After freezing, the ampules should be transferred to a liquid nitrogen-filled storage vessel. Prolonged storage at temperatures above -135°C will result in decreased viabilities.

3.4 Conclusion

You have studied the technique of cryopreservation and known by now the types of cells that can be cryopreserved for the purpose of reproduction. The steps to cryopreservation have also been shown.

Answer to Students' Assessment Exercise 3.1
Spermatozoa cells

3.5 Summary

Cultures to be cryopreserved should be healthy, free from contamination and should be maintained in log phase growth for several days before freezing. This technique is important in preserving germ cells over long periods of time.

3.6 Tutor-Marked Assignment

Elaborate on the concept of cryopreservation.

3.7 References and Further Reading

Engelmann, F.M.E., Dulloo, C., Astorga, S. Dussert and F. Anthony, edition (2007). Conserving Coffee Genetic Resources.(http//www.bioversityinternational.org/publication/pwfile.asp.ID-PUB=1224).

ReproTech Limited (2012). "Fertility Preservation" (http://reprotech.com/cryostorage/fertility-preservation/about-fertility-preservation html).

UNIT 4: ARTIFICIAL INSEMINATION

4.1 Introduction

This unit gives you a basic knowledge of what is meant by artificial insemination (AI) and its advantages and disadvantages. Some methods of semen collection from male animals for insemination into the female animals will also be discussed.

4.2 Objectives

By the time you have completed studying this unit, you should be able to:

- Define clearly what artificial insemination (AI) is;
- Highlight some advantages and disadvantages of AI;
- Be conversant with some common methods of semen collection;
- Have a knowledge of diluents used in semen extension and their composition.

4.3 Artificial Insemination (AI):

Is the possible impregnation of a female by artificial introduction of semen taken from a male. It is also defined as the process whereby semen collected from the male is artificially introduced into the female reproductive tract for the purposes of conception.

4.4 Advantages

- Eliminates time and space constraints or are taken off
- It is a very powerful tool for genetic development i.e. sex limitation on the part of the male is removed i.e. the best species of male is used for artificial insemination.
- There is no physical contact between male and females animals.
- It helps to control veneral disease spread in the animals, e.g. Brucellosis, etc. In artificial insemination, there is no physical contact between the male and female.
- It is more economical than in natural mating. The storage and preservation of semen is cheaper than keeping a bull alive. Greater economic value from a high quality bull by selling the semen.
- It helps in keeping accurate record of the female oestrous, thus breeding time can be determined. In artificial insemination, the bull to be used, the period of heat, time of breeding and time of parturition are all well recorded.
- It increases safety in the farm. The danger of keeping a bull is eliminated.

4.5 Disadvantages

- Conception rates are lower in artificial insemination than for natural mating.
- There is the need for accurate determination of oestrous and involves costs because it requires refrigeration facilities, with either liquid N_2 or solid CO_2 (dry ice).

  Liquid N_2 - at $-196°c$
  Dry Ice - at $-78°c$

  Liquid N_2 evaporates, so one has to keep topping up. Where Liquid N_2 is not readily available, forget about deep freezing.
- It requires good communication facilities to facilitate ease of contacting artificial insemination centres to inseminate animals.
- Artificial insemination comes into serious conflicts. Social norms and the production system does not permit easily, the introduction of exotic breeds.

Students' Assessment Exercise (SAE) 4.1
Question: Apart from the advantages of AI given above, which other do you know?

4.6 PROCESSES INVOLVED IN ARTIFICIAL INSEMINATION

- Conduct breeding program to determine the best males
- Have a lot of artificial insemination centres nationally

4.6.1 Semen Collection

Five methods can be used to collect semen in most farm animals.

a. The Artificial Vagina (AV) method.
b. The Dummy method
c. The electro ejaculator method
d. The Rectal massage method
e. The recovery method

a. The Artificial Vagina (AV) Method: The artificial vagina collection results in the best quality semen, therefore, of the five methods of collection, AV is the best. The AV is a special device that mimics the natural vagina. It consists of a hose opened at both ends. It has an inner lining held to both ends by rubber band. Water is introduced through the valve, and blown hot air. The valve is later closed or tightened and petroleum jelly rubbed to facilitate easy penetration of the male sex organ (mimic secretion of the natural vagina)

To collect semen, a teaser female or animal is tied to the collecting crate and the male whose semen is to be collected introduced. The male is allowed at least two false mounting before collection of the semen, by diverting his penis into the AV. The males have to be trained for collection of their semen through AV. Examples of collection from various species is given in table1.

b. The Dummy Method: This involves the design of a figure made in form of the animal, usually in pigs and a collecting tube inserted. The male whose semen is to be collected is trained to mount the dummy, ejaculate and the semen drained and collected in the collection tube. Semen collection using this method 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 locking-in of the penis in the cervix. The boar semen is released in distinct fractions sequentially as per-sperm, sperm rich and post sperm portions, which can be collected in 3 different collection flasks. Ejaculation in the boar lasts from 10 to 30 minutes.

c. Electro-ejaculation (EE): This method involves electrical probe. The electrical probe is inserted in the male's rectum after evacuating any feaces. The probe is 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 from EE is usually more dilute and poorer in quality than those from AV and dummy.

The male animal has to be well restrained during EE to contain any violent reaction arising from the electric shocks. Also, EE is said to shorten the life span of the males on which they are used.

d. The Rectal Massage Method: This involves insertion of hand by a person through the rectum, usually of a bull, when feaces have been evacuated. The region of the accessory sex glands is massaged to stimulate ejaculation, and the semen flows out through the sheath of the penis.

The quality of semen under this method is lower than the previous 3 earlier discussed, because of debris that normally follows the passing of semen though the sheath.

e. The Recovery Method: This involves insertion of vaginal peccaries into the vagina of the female animal on heat. The male is allowed to mount and ejaculate. The pessary inserted is withdrawn and semen squeezed out.
This method results in lower quality semen than the earlier 4 discussed.

Student's Assessment Exercise 4.2
Question: Which is the best method of semen collection?

4.6.2 Semen Evaluation

- **Appearance –** Normal appearance is creamy white. Blood stain includes injury or veneral disease.
- **Volume –** Should fall within range for the species.
- **Motility –** Good enough motility (70%) for storage is required. At X400mag, using a phase contrast microscope.
- **Concentration –** Should be within range for the species, determined using;
- Haemocytometer
- Electronic counting e.g. coulter counter
- Photo electric method

4.6.3 To Determine the Quality

- Live/dead counts – By use of eosin/nigrosin stain. They are supravital stains. Dead cells pick up eosin whereas live ones don't pick eosin.
- Morphology of the spermatozoa – can be determined using slide smears and immersion oil.
- pH – can also be determined using a pH meter.

Table 1: Ejaculate Characteristics of Some Farm Animals

	Farm Animals		
Trait	**Bull**	**Ram**	**Boar**
Volume (ml)	3-8	0.5-1.2	150-300
Sperm Conc. $\times 10^9$/ml	0.6-2	2-5	0.2-0.3
Motility %	60-85	60-90	60-80
Morphological Normal Spermatozoa	65-95	80-95	70-90
pH	6.9	6.9	7.5

4.6.4 Semen Storage/Preservation

There are several methods available, but common ones include:

1. Liquid Storage – This is storage above 0°C
2. Deep Frozen Storage – This is storage in about 196°C. Under this condition, they can live indefinitely.
3. Ambient Temperature Storage – From about 20-35°C. They survive for within 2 weeks.
4. Chilled Storage – Usually between 4-15°C. Under chilled condition, spermatozoa can survive for 6-7 days.
   Regardless of the storage method, the basic thing is to preserve the life of the cell where the spermatozoa will stay longer or to slow their metabolic rate.
   Under ambient temperature storage, provide a buffer and dilute the semen so that the waste product from semen metabolism does not accumulate fast.
5. Flows Dialysis Method – Fresh solution enters and the medium around the cells changes frequently. The semen and spermatozoa are inside the analysis bag, which is tied at both ends and then put inside the diluents solution. The diluents solution changed frequently at definite intervals, washing away sperm waste product and supplying fresh nutrients to the cells. Formaldehyde renders the spermatozoa temporarily dead, but after washing, phosphate buffered saline for some time, they became motile. First juice, milk etc are as with other diluents, but while milk preserves the cells, observing them under microscope becomes opaque and difficult because of fats. Under this method, cells survive for 8 days. When cocked in a container or McCartney bottles fig. 1 below (semen + diluents) they survive for 4 days.

6. Freeze Drying – Here, the water level of the spermatozoa are reduced to make it into a powdered form, but success for fertilization is poor under this method.
7. Other methods include formaldehyde preservation and fruit juice, milk, etc.formaldehyde render the spermatozoa temporarily dead, but other washing, phosphate buffered saline for some time, they become motile. First while move preserves the cells, observing them under microscope becomes opaque and difficult because of fats.

4.6.5 Principles of Semen Preservation

1. Slow down the metabolic rate – can be by reduction of temperature or use of chemical metabolic inhibitors e.g. Carbon dioxides $CO_{2,}$ Bicarbonates HCHO.

2. Eliminate injurious metabolic waste or by-products e.g. by dialysis, dilution, filtration or buffering.
3. Supply of energy source – e.g. fructose or glucose.
4. Maintenance of cell integrity to prevent cold shock especially in the case of deep freezing.
   This can be by use of egg yolk (protection against cold shock)
   - Albumin (prevent dilution shock)
   - Citrate (maintains membrane integrity and also a metabolite)
   - Cryoprotective agents (to prevent freezing injury) e.g. Glycerol, ethylene, glycol, erythritol.

4.6.6 Factors Affecting Fertility During AI

1. Method of semen collection and initial semen quality.
2. Species, breed and individual differences.
   Conception rate for cattle is higher than for sheep and goats, and boars.
   Cattle > Sheep and Goats > Pigs
3. Semen preservation method.
   Fresh or natural semen is better than chilled and chilled is better than frozen in terms of fertility. In terms of storage, frozen semen is better.
4. Processing method.
   - Holding time before process, the larger the holding time, before process the poorer and less fertility
   - Choice of diluents, some diluents are better infertility then others for a species
   - Dilution temperature – should beat about 30°C. Correct temperature will avoid shock or possible death leading to low fertility.
   - Dilution ratio, 1:4 (semen: diluents) best.
   - Method of dilution (mix gradually)
   - Diluents composition – should be such that pH is around 7; osmolarity should be about 300 milliosmol.
   - Cooling time to 5°C (should take 0.5-2hrs.). Outside normal range is not good for fertility.
   - Freezing method e.g.
     - Pellet freezing on dry ice
     - Straw freezing in liquid nitrogen vapour
   - Storage temperature – the lower the better in terms of length of time it can stay under storage, with some good fertility.
   - Length of storage (chilled or ambient temperature storage), the smaller the better.
   - Thawing temperature (should be 37°C)
   - Post thawing incubation period – the longer the poorer. Use immediately.
5. Timing of insemination.
   Generally, insemination should precede ovulation by a few hours. With single insemination, inseminate 12-24 hours after oestrus detection.
   For double insemination;
   - Ewes on heat detected in the morning, should be inseminated in the evening on same day and again on the following morning.

- Ewes on heat detected in the evening, should be inseminated in the morning and evening of the following day.

6. Insemination dose – This varies between 5million -500milloin, but in poultry, after evaluating sperm characteristics for quality, volume (in ml) is used for AI e.g. 0.05mI, 0.1mI or 0.2mI, etc.
7. Skill of the technician. Some AT technicians are better shocked; thereafter their work results in more fertility than others.
8. Types of Diluents - There are different types of diluents for extending semen as in table 1.6. Some diluents are better in preservingthe spermatozoa than others for sheep for instance, the Cornell University extender is better.
9. Methods and site of insemination – in cattle, an insemination gun is preferred and the site of insemination is the uterine harm. Inseminations in the vagina, cervix or even uterus results in lesser fertility compared to that of the uterine harm deposition. In sheep a vaginoscopeis used and semen deposited at the OS Cervix assigns a straw and syringe. For hens, the deposition is deep into the cloaca using a straw fitted to a syringe joined by a rubber band. In each case withdrawal of the straw or insemination gun should be gradual and gentle.

4.7 Composition

Ingredients	**Tris-Yolk**	**Cornell University Extender**	**Yolk Citrate**	**Homogenized Milk**
Tris* (g)	30.28	-	-	-
Citric acid (g)	16.75	0.87	-	-
Fructose (g)	12.50	-	-	-
$NaHCO_3$ (g)	-	2.10	-	-
KCL (g)	-	0.40	-	-
Tri-Na citrate (g)	-	14.50	29.0	-
Glucose (g)	-	3.0	-	-
Glysine (g)	-	9.37	-	-
Sulphanilamide (g)	-	3.0	-	-
Homogenized milk (cc)*[1] (Cow or Goat)	-	-	-	1000
Distilled water (cc)	-	To 1000	-	1000
Buffer (cc)	-	800	-	-
Egg yolk	200	200	-	-
Na penicillin (cu/ml)	1000	1000	1000	1000
Streptomycin Sulphate (mg/ml)	1	1	1	1
Distilled water	1000		1000	-

Types of Diluents

- Tris-yolk
- Cornell University Extender
- Homogenized Milk

*[1] Cow or goat milk, heated to 92°C for 10 minutes and cooled to 37°C.
* Tris -2 Amino – 2- (hydroxyl methyl) propane – 1, 3 – diol.

4.8 Conclusion

In this unit you have studied artificial (AI), the advantages and disadvantages of AI how to collect semen and evaluate as well as diluents types and composition.

Answer to Students' Assessment Exercise 4.1
Other advantages of AI are:

- Additional sources of employment
- Additional source of income for farmers

Answer to Students' Assessment Exercise 4.2
The best method of semen collection in terms of quality is the AV.

4.9 Summary

Artificial insemination (AI) has its advantages and disadvantages as well as methods of evaluating semen for AI. It is a powerful tool for genetic improvement.

4.10 Tutor-Marked Assignment

I. Discuss the factors affecting fertility during artificial insemination

4.11 Further Readings / Reference

- Osinowo O.A.2006. Introduction to Animal Reproduction. Sophie Academic Services Ltd. P.O Box 47, UNAAB Post Office, Abeokuta, Nigeria.
- Sorensen Jr. A.M. Repro Lab. A Laboratory Manual for Animal Reproduction. 3rd Edition. Kendall/Hunt Publishing Company, U.S.A.

MODULE 3: OESTRUS SYNCHRONIZATION AND PREGNANCY DIAGNOSIS

In this module you will be studying induction and synchronization of oestrus and ovulation in the first unit, while in the second unit under this module, you will study multiple ovulation and embryo transfer (ET) technique while the 3rd unit under this module deals with pregnancy diagnosis.

The objectives of the mode are:

i. To enable you understand what is meant by induction and synchronization of oestrus and ovulation

ii. To expose you to the issue of multiple ovulation and embryo transfer techniques.

iii. To enable you understand pregnancy diagnosis in farm animals.

UNIT 5: INDUCTION AND SYNCHRONIZATION OF OESTRUS AND OVULATION

5.0 Introduction

In this unit you will be studying about induction and synchronization of oestrus, its advantages and means of synchronization. Also control of ovulation, and its uses as means of improving livestock productivity will be studied.

5.1 Objectives

By the end of your studying this unit, you should be able to:

- State the advantages of oestrus synchronization;
- Describe the approaches to synchronization;
- List some means of synchronizing Oestrus

5.2 Synchronization of Oestrus

Is a term used to indicate the process of bringing groups of animals into heat together in response to some form of treatment.Such animals should therefore conceive at closely similar times, proceed through pregnancy together and produce their offsprings in or within a short period.

5.2.1 Advantages

1. It enables regulation of time of heat and possibly ovulation
2. Enables uniform group feeding, supervision, cross fostering, batch weaning, fattening and marketing.
3. Enables rationalization of the use of labour, buildings and other resources.

5.2.2 Approaches to Synchronization

Synchronization of oestrus involves 2 approaches

1. Inducing regression of the corpus luteum (CL) so that all animals in an appropriate group enter the follicular phase and return to oestrus at a closely similar time.
2. Suppression of ovarian follicular development so that after removing the hormonal or pharmacological blockade, animals rebound into a compact follicular phase followed by a synchronized oestrus.

5.2.3 Means of Synchronizing Oestrus

The means of synchronizing oestrus is varied and has been mostly done for cattle but the trend applies to all animals with varied successes. They include:

1. Injection of a solution of progesterone (to mimic the activity of the CL).
2. Feeding synthetic forms of progesterone (i.e. oral progestagens).
3. Implanting silicone rubber capsules of progesterone under the skin.
4. Inserting intra-vaginal sponges or coils containing progestagens.
5. Use of $PGF_{2\alpha}$ or analogues of $PGF_{2\alpha}$ to cause spontaneous regression of CL. Their response to $PGF_{2\alpha}$ will depend on the stage in their cycle. Those with a CL younger than five days or those already in the follicular phase, will not respond to the injection. However, a second injection given 10-12 days later

should find 90-95% animals with mature CL sensitive to the lytic influence of $PGF_{2\alpha}$. The animals return to heat 2-3 days after the injection.

5.3 Control of Ovulation

Control of ovulation aims to regulate the precise time of ovulation and/or the number of follicles ovulating. These objectives are achieved most directly by injection of gonadotropic hormones.

5.3.1 Advantages

1. Enables AI at the optimum time, with or without detection of oestrus
2. Improves conception rates and avoids the deleterious effects of aging of the gametes.
3. Enables accurate calculation of the time of fertilization and developmental stage of embryos, which are necessary in transplantation studies.

5.3.2 Hormone Preparation

Hormone preparations for influencing ovarian follicular development fall into two main categories:

a. Those rich in follicle stimulating hormone (FSH) like activity e.g. Pregnant Mare Serum Gonadotropin (PMSG) – should be used at onset of follicular phase.
b. Those rich in LH like activity e.g. Human Chorionic Gonadotropin (hCG) – should be used at proestrus.

If the response to the injection in terms of the number of follicles ovulating is significantly above the normal ovulation rate for the species or breed in question, then this is referred to as Superovulation.

Time of ovulation refers to the moment of follicular collapse with release of egg(s).

Student self-Assessment Exercise (SAE) 5.1

What is aim of the control of Ovulation?

Conclusion

In this unit, you have studied synchronization of Oestrus and ovulation. The advantages have been spelt out as well as some means of synchronizing oestrus in farm animals.

Students' Assessment Exercise 3.2

Question: State any two advantages of embryo transplantation

Answers to Students' Assessment Exercise 3.1

Synchronization of oestrus is a term used to indicate the process of bringing groups of animals into heat together in response to some form of treatment.

Students' Assessment Exercise 3.2

Some two advantages of embryo transfer are:

- Rapidly multiplying up the number of offspring from a pedigree or exotic donor.
- Shortening the generation interval by breeding from prepubetal females

5.11 Tutor-Marked Assignment

Describe the process of embryo transplantation

5.12 References

Hunter R.H.F. 1979. Reproduction of Farm Animals.Sorensen Jr. A.M. Repro Lab. A Laboratory Manual for Animal Reproduction.3rd Edition.
Kendall/Hunt Publishing Company, U.S.A.

UNIT 6: INDUCTION AND SYNCHRONIZATION OF OESTRUS AND OVULATION; MULTIPLE OVULATION AND EMBRYO TRANSFER TECHNIQUE

6.0 Introduction

In this unit, you will be studying multiple ovulations, embryo transfer and its uses as means of improving livestock productivity.

6.1 Objectives

By the end of your studying this unit, you should be able to:

- State the advantages of oestrus synchronization;
- Describe the technique of embryo transplantation and storage;
- State the uses of embryo transplantation.

6.2 Synchronization of Oestrus

Is a term used to indicate the process of bringing groups of animals into heat together in response to some form of treatment. Such animals should therefore conceive at closely similar times, proceed through pregnancy together and produce their offsprings in a compact period.

6.2.1 Advantages

1. It enables regulation of time of heat and possibly ovulation
2. Enables uniform group feeding, supervision, cross fostering, batch weaning, fattening and marketing.
3. Enables rationalization of the use of labour, buildings and other resources.

6.2.2 Approaches to Synchronization

Synchronization of oestrus involves 2 approaches

1. Inducing regression of the corpus luteum (CL) so that all animals in an appropriate group enter the follicular phase and return to oestrus at a closely similar time.
2. Suppression of ovarian follicular development so that after removing the hormonal or pharmacological blockade, animals rebound into a compact follicular phase followed by a synchronized oestrus.

6.2.3 Means of Synchronizing Oestrus

The means of synchronizing oestrus is varied and has been mostly done for cattle but the trend applies to all animals with varied successes. They include:

1. Injection of a solution of progesterone (to mimic the activity of the CL).
2. Feeding synthetic forms of progesterone (i.e. oral progestagens).
3. Implanting silicone rubber capsules of progesterone under the skin.
4. Inserting intra-vaginal sponges or coils containing progestagens.
5. Use of $PGF_{2\alpha}$ or analogues of $PGF_{2\alpha}$ to cause spontaneous regression of CL. Their response to $PGF_{2\alpha}$ will depend on the stage in their cycle. Those with a CL younger than five days or those already in the follicular phase, will not respond to the injection. However, a second injection given 10-12 days later

should find 90-95% animals with mature CL sensitive to the lytic influence of $PGF_{2\alpha}$. The animals return to heat 2-3 days after the injection.

6.3 Control of Ovulation

Control of ovulation aims to regulate the precise time of ovulation and/or the number of follicles ovulating. These objectives are achieved most directly by injection of gonadotropic hormones.

6.3.1 Advantages

1. Enables AI at the optimum time, with or without detection of oestrus
2. Improves conception rates and avoids the deleterious effects of aging of the gametes.
3. Enables accurate calculation of the time of fertilization and developmental stage of embryos, which are necessary in transplantation studies.

6.3.2 Hormone Preparation

Hormone preparations for influencing ovarian follicular development fall into two main categories:

a. Those rich in follicle stimulating hormone (FSH) like activity e.g. Pregnant Mare Serum Gonadotropin (PMSG) – should be at onset of follicular phase.
b. Those rich in LH like activity e.g. Human Chromonic Gonadotropin (hCG) – should be at proestrus.

If the response to the injection in terms of the number of follicles ovulating is significantly above the normal ovulation rate for the species or breed in question, then this is referred to as Superovulation.

Time of ovulation refers to the moment of follicular collapse with release of egg(s).

6.4 Embryo Transplantation and Storage

This technique requires recovery of embryos by flushing fluid through the reproductive tract of the donor animal (which may or may not have been super ovulated), examination of the embryos under a binocular microscope, and then their insertion into the reproductive tract of the recipient or foster mother using a glass pipette.

The recovery and transplantation procedure may involve abdominal surgery under full or local anesthesia, or they may gain access to the uterus of conscious animals through the vagina and cervix as in AI.

6.5 Supply of Embryos

A supply of suitable embryos is essential and procedures of superovulation are usually applied to the donor animal. The superovulated donor is bred naturally or if by AI with an increased number of spermatozoa and usually 2 or 3 inseminations.

Embryos may be transplanted in the fresh condition shortly after examination under a dissecting microscope, or they may be used after storage on a temporary or long-term basis.

6.6 Synchronization of Donor and Recipient

Successful transplantation of embryos depends on close synchronization of the oestrous cycles of donor and recipient animals, for 2 reasons:

a. Growth of the embryo is tightly programmed in terms of its requirements from the uterine secretions.
b. The chemical nature of these secretions is changing progressively with the time elapsing from ovulation.

In practice, ±1 day in cattle ±2 days in sheep are compatible with establishment of pregnancy.

Students' Assessment Exercise 3.1
Question: Define synchronization of oestrus

6.7 Site of Recovery and Transfer

Although embryos can be recovered from the oviducts during abdominal surgery, it is more convenient in most circumstances to wait until the embryos have entered the uterus, 3 or so days after ovulation. Recovery can be by surgical or non-surgical procedures. In either case, the uterine lumen is flushed with a sterile physiological solution that is collected through a rubber catheter with a balloon cuff into a round-bottomed glass dish. A physiological medium is introduced through the catheter, flushed around the uterine horn, and returns through the catheter to a collection dish.

If a single embryo is being transplanted to an unbred recipient, it must be introduced into the horn of the uterus adjoining the ovary with a Corpus Luteum (CL). If an embryo is being added to an animal already mated or inseminated, then this is deposited in the uterine horn opposite the ovary with the CL. In the case of transplantation of two embryos, it is essential to deposit one per horn, since there is little intra-uterine migration in cattle, and competition between growing embryos may lead to death of one or both.

6.8 Uses of Embryo Transplantation

1. Rapidly multiplying up the number of offspring from a pedigree or exotic donor.
2. Increasing total calf output by means of twinning - the heritability of twinning being less than 10%.
3. Produces more calves from best cows in short period i.e. more female calves of high value and better bulls for AI centres.
4. Rapid multiplication of rare or commercially desirable breeds
5. In conjunction with superovulation, to speed up selection programmes.
6. To induce twinning i.e. either transplanting 2 embryos to unmated recipients or by adding one embryo to a mated recipient.
7. As part of an export programme to upgrade stock in developing countries.
8. To shorten the generation interval by breeding from prepuberal females.
9. As an experimental procedure, after deep-freezing, cloning or sexing of embryos.

Students' Assessment Exercise 3.2
Question: State any two advantages of embryo transplantation

6.9 Conclusion

You have studied one of the main techniques for improving livestock productivity (embryo transfer) especially in large ruminants like cattle. The advantages of this technique have also been shown.

Answers to Students' Assessment Exercise 3.1
Synchronization of oestrus is a term used to indicate the process of bringing groups of animals into heat together in response to some form of treatment.

Students' Assessment Exercise 3.2
Some two advantages of embryo transfer are:

- Rapidly multiplying up the number of offspring from a pedigree or exotic donor.
- Shortening the generation interval by breeding from prepuberal females

6.10 Summary

Embryo transplantation requires the recovery of embryos by flushing fluid through the reproductive tract of the donor animal (which may or may not have been superovulated) examination of the embryos under a binocular microscope, and then their insertion into the reproductive tract of the recipient or foster mother using a glass pipette. This technique is important in multiplying offspring of parents with desired traits. The process of this technique has been shown in this unit.

6.11 Tutor-Marked Assignment

Describe the process of embryo transplantation

6.12 References

Hunter R.H.F. 1979. Reproduction of Farm Animals.Sorensen Jr. A.M. Repro Lab. A Laboratory Manual for Animal Reproduction.3rd Edition.
Kendall/Hunt Publishing Company, U.S.A.

UNIT 7: PREGNANCY DIAGNOSIS

7.0 Introduction

In this unit you will be studying pregnancy diagnosis in farm animals. The diagnosis of pregnancy requires a multifaceted approach, which you will discover in the course of your study in this unit. Successful pregnancy and subsequent delivery is crucial for any profitable livestock farming.

7.1 Objectives

At the end of your studying this unit, you should be able to:

- List the 3 main pregnancy diagnostic tools;
- Describe the visual methods;
- List the clinical methods;
- List some other methods.

7.2 Pregnancy Diagnosis

The diagnosis of pregnancy requires a multifaceted approach using 3 main diagnostic tools. These are history and physical examination laboratory evaluation, and ultrasonography.

Visual methods are far from perfect in domestic animals. In animals like cattle, buffaloes and mares' recto genital palpation and trans-rectal ultrasonography continue to be the methods of choice for an accurate and early pregnancy diagnosis. In sheep, goat, sow, bitch and cat ultrasonography is the only reliable method of pregnancy diagnosis. In the camel, cocking of the tail is an effective visual method of pregnancy diagnosis and recto genital and ultrasonography are also useful. In rabbits, palpation of the abdomen in pregnant does has maize grains like feeling by mid gestation period.

7.3 Visual Methods

7.3.1 Non Return to Oestrus

When an animal is mated or inseminated and it does not return to oestrus, the owner usually thinks that the animal has become pregnant. However, many a times the animal does not return to oestrus because of non-regression of CL due to reasons other than pregnancy.

7.3.2 Cocking of the Tail

The pregnant female dromedary camels exhibit a characteristic behavior when approached by a male or a person. The female assumes a stiffened posture with the head held high and tail curled upwards. This is known as cocking of the tail. This behavior appears 14 to 15 days after fertile mating and known to be 95% reliable for pregnancy diagnosis in quiet and calm dromedary female camels. However, many false positives can be obtained in agitated females if the observer is untrained. Tail cocking is also observed in the pregnant bactenian camel although not with the same intensity as in the dromedary female camel.

7.4 Clinical Methods Of Pregnancy Diagnosis

Four clinical methods are available for farm and pet animal species.

1. Rectal Palpation
2. Abdominal ballottement
3. Ultrasonography
4. Radiography

7.4.1 Rectal Palpation:

Transrectal palpation is the oldest and most widely used method for early pregnancy diagnosis in dairy cattle. In most large domestic animal species like cattle, buffaloes, mares and female camels, recto-genital palpation (with some limitation) is the easiest, cheapest and fastest method of pregnancy diagnosis with little or nil harm to the animal and its fetus when performed carefully. To a limited extent, this method is used for pregnancy diagnosis in pigs.

Precautions during rectal palpation.

a. Ruthless movements of the hand in the rectum should be avoided.
b. Examiners must trim nails and avoid using dirty soiled sleeves.
c. Rectal examination without sleeve must be avoided especially in mares to avoid contracting diseases and obnoxious odors.
d. Rectal examination of animals suffering from sever should be extremely gentle or better avoided as the blood vessels are more fragile and bleed easily.
e. Compared to cattle, rectal palpation in buffaloes must be gentle as the rectal mucosa is more fragile and bleed easily.
f. Uncareful palpation of the uterine horns with undue pressure can cause rupture of the amniotic vesicle and loss of an early pregnancy and hence this must be avoided.

7.4.2 Abdominal Ballottement:

This involves palpation to determine pregnancy by pushing up against the uterus with the finger so as to feel any downward pressure exerted by an embryo as it sinks back into place in the amniotic fluid. In cows this can be done at 7m and more

7.4.3 Ultrasonography

Ultrasonography is a high frequency sound wave. Ultrasonography has gained popularity in veterinary medicine and some reproductive physiology and has become the method of choice for diagnostic imaging of the various organs of the body, including reproductive organs.

The ultrasound equipment basically consists of a transducer and a scan converter. The transducer is the ultrasound producing part. The ultrasound is transmitted to the patient from the transducer and propagates through the tissues. Trained personnel are needed to interpret the variations in brightness displayed. Different instruments are used in ultrasonography.

Sonographic Findings during Pregnancy

Interpretation of sonograms of the reproductive tract requires an understanding of the composition of the images and an awareness of the possible artifacts which can occur and lead to misdiagnosis.

7.4.4 Radiography

To a limited extent, radiography has been used for pregnancy diagnosis in the small ruminants (sheep and goat), the companion animals (dog and cat) and rarely in pigs. The technique is known to be good in evaluating fetal numbers in the bitch and cat, but is poor in evaluating fetal viability. It uses X-rays, therefore risky to the fetus, needs expertise and is costly.

7.5 Others:

7.5.1 Fetal Echocardiography

To a limited extent, fetal echocardiography has been used in the past to diagnose in cattle, sheep and mares, but with the advent of ultrasonography its use has been limited.

7.5.2 Vaginal Electrical Resistance

The conductivity of the vaginal mucous membrane changes at oestrus due to increased hydration increased blood supply and other changes. When measured by ohm meters,

the vaginal electric resistance (VER) is low at oestrus. Hence when VER is measured constantly, animals returning to oestrus can be identified and thus those probably becoming pregnant can be differentiated but mistakes are common.

7.5.3 Laparoscopy

Laparoscopy can be used as a method of pregnancy diagnosis by directly visualizing the genitalia in animals however, the invasive nature of the technique, the high cost of equipment and clinic required, and the availability of non-invasive techniques limits the use of this technique as a means of pregnancy diagnosis in most animals.

7.6 Laboratory Tests For Pregnancy Diagnosis

The various laboratory tests developed for pregnancy diagnosis in domestic animals are indirect methods of pregnancy evaluation and utilize qualitative or quantitative measures of reproductive hormones at specific stages after AI or mating or detect conceptus specific substances in maternal body parts or body fluid as indirect indicators of the presence of a viable pregnancy. Unfortunately, none of the methods developed so far in animals are as accurate as is the detection of hCG in pregnant human females. However, the research to develop commercial indirect methods continues because these methods are non evasive and the tests can be marketed to and performed by dairy farmers. The currently available methods are briefly described.

7.6.1 Progesterone hormone assay:

The corpus luteum formed on the ovary subsequent to ovulation produces progesterone for maintenance of pregnancy for a reasonable period in some species and for entire gestation in the species like the cow, buffalo, goat and sow. The elevated progesterone levels are used as means of pregnancy diagnosis.

7.6.2 Estrone sulfate

The estrone sulfate is produced by the fetomaternal axis or the conceptus and therefore its presence in urine, milk, feces or blood is an indicator of pregnancy. The detection of these hormones depends on availability of appropriate kits and personnel.

7.7 Chemical Tests For Pregnancy Diagnosis

Most chemical tests reported in the past appear to be of historic importance only in current day pregnancy diagnostic procedures. Some of the chemical tests that utilize urinary estrogens or other molecules as a basis of pregnancy diagnosis in domestic animals are described below:

7.7.1 Cuboni Test

This test was first developed by cuboni (1934) and modified later (Galina and COX, 1969). The test is performed in the mare for detection of pregnancy through assay of urinary conjugated estrogens.

The cuboni test is only effective beyond 150 days of gestation, and also predicts fetal viability.

7.7.1 The Mouse Test

In the mouse test, the serum or urine from pregnant mares when injected to ovariectomized mouse or rats would induce vaginal edema, appearance of cornified

cells and mucus discharge due to presence of estrogens in the pregnant mare's serum or urine.

There are many other tests available.

Some other previously described tests for pregnancy diagnosis in cows include two tests on milk (1) milk alcohol coagulation test: In this test, there is coagulation of milk from pregnant cows when mixed with equal quantities of alcohol and allowed to stand for 1-3 hours. (2) Copper Sulfate test. 1ml of milk when mixed with a few drops of 3% copper sulfate coagulates if the animal is pregnant.

Kosjakov's Test
This test apprehends that the sulfur content of hair in pregnant animals is increased. A few other tests exist.

7.8 Assay Of Gonadotrophins

The human female secretes the gonadotropin hCG which is present in sufficient quantities in the urine of pregnant women and many simplified tests have been developed to detect this molecule in urine for an easy pregnancy diagnosis in women. The eCG continues to be secreted from day 40 to 120 days of pregnancy and is the basis of tests currently available on farm.

7.9 Biologic Tests

Several biologic tests were developed for the detection of eCG including the AschiemZondek Test, the Friedman test (rabbit test) or the frog or toad test.

In AschiemZondek test – A woman's urine is injected into an immature rat or mouse. If the subject was not pregnant, there would be no reaction. In the case of pregnancy, the rat would show an oestrous reaction (be in heat) despite its immaturity. This test implied that during pregnancy there was an increased production of the hormone.

7.10 Friedman Rabbit Test

Serum from test mare is injected (2ml given IV) to rabbits (14 to 20 weeks of age) kept in isolation and laparotomy performed 24 hours later. A positive test is indicated by the presence of corpus haemorrhagicum and uterine edema.

Toad Test – The basis of this test is the concept that the sperm cells are emitted by toads/frogs only when stimulated with female frogs or gonadotrophins.

Many of the above older methods have now been replaced by newer methods including radioimmuno assay, radioreceptor assay, haemagglutination inhibition test, ELISA and indirect latex agglutination tests. Commercially available kits are in use at many places for these assays.

Pregnamare (R) is one such test, which can be performed on blood between day 40 and 100 of pregnancy, however, false results may be obtained if fetal death occurs after formation of the endometrial cups.

7.10 Other Methods

7.11.1 Cervical Mucus

Some years ago, a pregnancy diagnosis based in determining differences in cervical mucus using nuclear magnetic resonance was used (Merilan,1983). The test looked promising at that time but no subsequent information is available.

7.11.2 Milk ejection by low dose prostaglandin

This is a method tested some years ago involving the injection of low does breeding resulting into milk ejection. The animals detected further as pregnant showed an increase in the pressure in the milk ejection and alveolar milk volume collected by a test probe in comparison with the non-pregnant cows.

However, due to potential dangers of inducing lubeolysis by accidental over dosage, the use of this technique of pregnancy diagnosis, could not gain wide popularity.

7.11.3 Pregnancy associated glycoprotein (PAG)

Pregnancy specific proteins are known to be produced in various ruminant species including cattle, buffalo, sheep and goats. Recently too, the existence of PAG has been documented in bovine milk.

7.11.4 Early pregnancy factor (EPF)

This protein molecule was first identified in pregnant mice and later in sheep and cattle by using the rosette inhibition bioassay.

7.11.5 Relaxin assay

Relaxin can be determined in the peripheral circulation of pregnant bitches at 20-30 days of gestation, whereas it is absent in non-pregnant bitches at all stages of the reproductive cycle.

7.11.6 Vaginal biopsy

Historical assessment of the number of layers of the stratified squamous epithelium of the vaginal mucosa obtained by biopsy can be used as a method of diagnosing pregnancy in the sow. The basis for the test is the decrease in the layers of stratum germinativum(vaginal epithelium cells: 3 to 4 layers at 18 – 25 days of pregnancy) under the unfluence of progesterone (P_4). The number of layers is high at oestrus (around 20 layers) due to influence of oestrogen hormones. It is also done in sheep after 40 days of pregnancy.

With the availability of more precise techniques of pregnancy diagnosis in sheep and sow, the use of vaginal biopsy for pregnancy diagnosis has been reduced.

Students' Assessment Exercise 6.1

- List the 3 main pregnancy diagnosis tools

Students' Assessment Exercise 6.2

- List clinical methods of pregnancy diagnosis

7.12 Conclusion

Detection of pregnancy on animals that have successfully conceived is part of good management practices to reduce cost and enhance productivity. Each farmer should be able to employ some of the methods of pregnancy diagnosis explained in this unit.

Answers to Students' Assessment Exercise 6.1
The three tools are:

1. History
2. Physical examination
3. Laboratory examination

Answers to Students' Assessment Exercise 6.2
The 4 clinical methods of pregnancy diagnosis are:

1. Rectal palpation
2. Abdominal ballottement
3. Ultrasonography
4. Radiography

7.13 Summary

Pregnancy diagnosis tools have been shown in this unit. The various methods are such that a good farm manager should be able to employ some to his own benefits.

7.14 Tutor-Marked Assignments

7.15 References

- Adams C.S., Jardon P.W. (1999). Evaluation of the Early Conception Factor Test in Cows 3-7 Days Post Breeding. Roc. Am. Assoc. Bov. Pract. 32:340-241
- White JR, Russel AJF, Wright A. (1985). Real Time Ultrasonic Scanning in the Diagnosis of Pregnancy and the Estimation of Gestation age in Cattle. Vet. Rec. 117:5
- Zemjanis R. (1970). Pregnancy Examination: Diagnostic and Therapeutic Techniques in Animal Reproduction. 2nd ed. 29: Baltimore: Williams and Wilkins.

MODULE 4: PARTURITION AND LACTATION

Introduction

In this module you have two units. The first unit deals with the issue of induction of parturition, while the second unit deals with the issue of lactation.

These objectives are:

i. To enable you understand how parturition is induced in farm animals
ii. To enable understand lactation as a consequence of reproduction.

UNIT 8: INDUCTION OF PARTURITION

8.0 Introduction

In this unit you will be studying methods of induction of parturition and how it is achieved, indicators and hormonal tredtments. You will also learn that there are already either therapeutic or management issues for inducing parturition, and it should be embarked upon as a last resort.

At the end of this unit, you should be able to:

- List dome methods of inducing parturition.
- List some recommendations.
- List some induction of parturition indications.
- List some important criteria for successful induction of parturition

8.1 Induction of Parturition

The induction of parturition can be broadly divided into therapeutic and managemental. Of the later, the most common are induction of abortion in early gestation (usually to terminate unwanted pregnancies) and induction of parturition close to full – term (as a means of preventing excessive foetal growth associated with gestations prolonged beyond 282 days in the cow. It has been suggested that this could also be used as a management tool to synchromise parturition. However, in a number of cases where parturition is induced, there is the risk of retention of the placenta (after birth) which may lead to infection of the uterus, possible future reproductive failure and loss of milk production. Induction of parturition should therefore be avoided unless the welfare of the cow or calf is likely to be improved.

8.2 Recommendations

1. Animals should not be mated too young or mated by an inappropriate sire.
2. The induction of parturition should never be used as a routine procedure. Correct nutrition and sire selection should be the first option and will minimize the need for this technique. However, as a last resort, it may be considered as a way of preventing cows from having to deliver grossly oversized calves.
3. Induction of parturition should not be used as a management tool if this involves induction well before full-term and production of premature, unviable calves.

8.3 Induction of Parturition Indications

1. Managemental causes as you desire that animal should participate in competition. Consider the husbandry facilities, availability of labor, and man power. So we induce parturition to make the use of maximum facilities.
2. Health conditions – health of dam is deteriorating day by day so induce the parturition. Induce parturition two weeks before normal due date; otherwise less survivability of fetus.
3. To avoid dystocia because during last two weeks growth of fetus is enhanced and daily weight gain of fetus is increased.
4. To avoid udder injuries or excessive udder edema as development of udder near the parturition is fast.
5. Truncate the calving season (Cut – short). There by allowing more time post-partum to resume cyclicity before the next breeding season.

8.4 Some Important criteria for successful induction of parturition.

1. The method must be effective.
2. Must have a predictable time (less variation).
3. Treatment should not have any adverse effect on dam and on health of the calve.
4. Method should not affect the quality nor the quantity of colostrum because immunoglobulins are transferred quickly from blood to milk in udder during last two weeks. Colostrum is only the source of immunity for the calf.
5. Post-partum involution and subsequent fertility is not affected.
6. It should not increase the incidence of retained placenta.

8.5 Precautions

1. Accurate and reliable breeding record should be known. Exact date of breeding should be known.
2. Must consider the facilities, manpower, husbandry and life span etc.

8.6 Treatment

Hormonal treatment;

1. Prostaglandins.
2. Corticosteroids.
3. Estrogen.
4. Combination of these.

Student's Self-Assessment Exercise 8.1
List any three (3) hormones for inducing parturition.

Use of Long Acting corticosteroids.

These are given intramuscularly one month before the due date of parturition and parturition occurs between 4 -26 days. Not good method; wider range of time is the disadvantage. Here the incidence of RFM (Retained fetal membrane) is less. High incidence of calf mortality 45 – 70% due to premature placenta separation and uterine inertia.Calf died in uterus and born autolysed. Calf lacks the immunoglobulins. Calf is weak and cannot ingest adequate amount of colostrum.

Short Acting Corticosteroids:

Efficacy is 80 – 100%. Give intramuscularly two weeks before due date, parturition may occur within 24 -72 hours with an average of 48 hours. Amount of IGs (immunoglobulins) is normal in colostrum. It increases the incidence of retained placenta which is related to degree of prematurity. Incidence range is 30 – 100%.

Use of Prostaglandins:

Use of prostaglandins is similar to corticosteroids short acting. Its efficacy is similar to short acting corticosteroids. So no advantage over corticosteroids.

Use of Estrogens:

Use of estrogen is considered as old method (before the availability of PGF_{2x}). Disadvantage is poor efficacy and high incidence of retention of fetal membrane combination of short Acting Corticosteroids + Estrogen:
It does not decrease the incidence of retained placenta. However, larger doses of estrogen decrease the interval of parturition by several hours. Chances of failure are lower.

Long Acting + short Acting/PGF_{2x}:

After administration of 7 – 12 days of long acting, the short acting PGF_{2x} is given then at 2 – 3 days before parturition occurs. Interval is short and predictable; however, incidence of calf mortality is high. Corticosteroids and PGF_{2x} does not decrease percentage of retained but chances of failure are decreased.

8.7 Conclusion

In this unit, you have studied induction of parturition either as a management tool or for health reasons. The recommendations and induction of parturition indicators were also studied, as well as hormone treatment or their combinations to induce parturition.

Answer to Student's Assessment Exercise 8.1
Three hormones for the induction of parturition will include;

1. Prostaglandins.
2. Corticorsteroids.
3. Estrogens.
4. Combinations of these.

8.8 Summary

The induction of parturition can be broadly divided into theurapeutic and managemental. Induction of parturition should be the last option, only when other management steps like nutrition, correct size combinations (sire and dam), have failed. Indications for induction of parturition and the availability of both facilities and personnel must be clearly certain before embarking on this procedure.

8.9 Tutor Marked Assignment

Elaborate on some induction of parturition indicators.

9.0 References

1. Claydon R. K. 1984. Induction of Parturition in Cattle During the later Stages of Pregnancy Veterinary – Record.6mj.com>volume114, issue 5.

2. Peters A. R. 1992. Induction of Parturition in Dairy cows with Dexamethasone. Veterinary Record, 1992 – 171 . 67. 117. 160.

3. www.fawc.org.uk/reports/dairycow/dcowsro53.htm.Induction of Parturition.Accessed February, 2014.

Answer to TMA 8.10

Question:
Elaborate on some induction of parturition indicators.

Answer:
Induction of Parturition should only be embarked upon as a last resort. Induction of parturition indicators include;

i. Managemental causes as you desire that animal should participate in competition.
ii. Health conditions. Where health of dam is deteriorating. If induction is done two weeks to date of normal birth, survivability is better.
iii. To avoid dystocia because during enhanced and daily weight gain of fetus is increased.
iv. To avoid udder injuries or excessive udder edema.
v. Truncate the calving season, thereby allowing more time post partum to resume cyclicity before the next breeding season.

UNIT 9 LACTATION

9.0 Introduction

In this unit you will be studying lactation as well as weaning and various weaning methods in farm animals.

9.1 Objectives

At the end of the study in this unit, you should be able to:

- List some methods of inducting lactation
- List some methods of weaning

9.2 Lactation

Milk ejection and lactation are consequence of parturition. Milk ejection can be induced by low dose prostaglandin. Injection of low dose prostaglandin F2 alpha

(PGf22) (non-luteolytic dose) in animals two weeks after breeding results in milk ejection. The intrajugular administration of a subluteolytic dose of $PGF_{2\alpha}$ induces a large increase in intramammary pressure when given during the luteal phase and this is directly correlated to the plasma progesterone (P_4) profiles.

Removal of the prolactin inhibiting factors also results in milk ejection and lactation.

9.3 Weaning Methods

Weaning refers to the process of causing the young to become accustomed gradually to food other than its mother's milk.

It is important to decide when and by what means to wean calves, because it influences the weaning mass of calves as well as the condition of the cows and indirectly their conception rates.

9.3.1 Timing

- The major priority in beef cattle production is to produce as many calves as possible. The main objective of weaning is therefore to enable a cow to calve every year by allowing her to regain condition after weaning.
- Calves are ideally weaned when they are 7 to 8 months old.
- The right time to wean a calf depends on the condition of the cow and not the age of the calf.
- Calves should be weaned before the condition score of the cow falls below 2-5 if adequate feed is available and the cows maintain their condition. The calves should preferably be weaned before the cow's condition score falls below 3.0.
- During years of draught and poor feed supply, calves should be weaned early (about 6 months), to allow the cow to recover before the onset of winter or harmattan.
- It is important that the cow should recover and that secretory tissue be restored before the next calf is born.

9.3.2 Early Weaning

- This practice should only be considered during times of severe drought or feed shortages.
- Calves weaned at a relatively young age (less than 5 months) experience severe setbacks.
- If the condition of the cow deteriorates considerably before the planned weaning time, the producer must decide whether to:
    - Wean early and supply concentrate feeding to the calf.
    - Provide a roughage supplement to the cows that are still suckling their calves.
- This decision will depend on the availability and cost of feed. Generally, the feed (mainly concentrates) costs to rear early – weaned calves are relatively high. Therefore, feeding concentrates to calves should only be considered during adverse conditions.

9.4 Methods of Weaning

Circumstances on the farm determine the method of weaning. The following methods can be used:

1. Keep the calves in a kraal or well-fenced camp and remove the cows to a distant camp, preferably out of earshot of the calves.
2. Remove the cows temporarily from a camp and in their absence move the calves to another distant camp. Cows tend to look for their calves in the camp in which they were last seen and this method should prevent the cows from breaking out of the camp.
3. Exchange calves from two different herds. The calves will then have the company of cows. Some cross-suckling is however, likely to occur.
4. Separate the cows and calves by a strong, close-strand wire fence. This method can reduce weaning stress
5. Nose plates, commercially available or home-made can be fitted to calves for 7 to 14 days. These prevent suckling, even if cows and calves remain together throughout the weaning period. When the nose plates are removed the cows and calves are separated, but with relatively little stress.

Students' Assessment Exercise 7.2
Question: Describe the method of weaning in cattle production.

9.5 General

- Perform castration, dehorning and branding when calves are 2 to 3 months old, not immediately before weaning. This will ensure that the stress associated with these operations does not add to that of weaning.
- A few dry cows can be kept with the weanere to calm them.
- Provide sufficient good-quality roughage, water and shade in the weaning camps. To prevent excessive walking and trampling the camps should not be too large.
- The weaning process could last 7 to 14 days, depending on the age at which the calves are weaned as well as the breed of the cow.

9.6 Conclusion

In this unit, you have studied lactation and how to induce it. You also studied weaning methods, and other general management practices for …….., such as castration, dehorning and branding were mentioned.

9.7 Summary

Milk ejection and lactation are ordinary consequences of parturition. However, milk ejection can be induced by low dose pasta landing; removal of the prolactin inhabiting factors also results in milk ejection and lactation.

Weaning refers to the process of causing the young to become accustomed gradually to food other than its mother's milk. Circumstances on the farm determine the method of weaning.

Answer to Students' Assessment Exercise 8.1
Estrogen is used in various induction regimes.

Answer to Students' Assessment Exercise 9.2
One method of weaning in cattle production is to keep the calves in a kraal or well-fenced camp and remove the cows to a distant camp, preferably out of earshot of the calves.

9.6 Tutor-Marked Assignment

Write short notes on any 3 methods of weaning.

9.7 References

- Tominson C., Marshall J., Ellis J.E. Comparison of Accuracy and Certain of Results of Six Home Pregnancy Tests Available Over-the-Counter. Carr. Med. Res. Opin. June 2008 24(6) 184-9 (Medicine).
- Andrea D. Shields (2012). Pregnancy Diagnosis. http://emedicine.meds-cape.com/article 262591-overview.

MODULE 5 INVITRO-MATURATION, FERTILIZATION AND CLONING

Introduction

In this module, there are two units. The first unit under this module deals with the topic of invitro-maturation and fertilization while the last unit or second unit under the module deals with the issue of cloning.

The objectives of the module are

i To enlighten you on the concept of invitro-maturation

ii To enlighten you on invitro fertilization

iii To enlighten you on concept of cloning

UNIT 10: INVITRO-MATURATION, FERTILIZATION AND CLONING

10.0 Introduction

In this unit, you will be studying about some more recent techniques in reproduction, such as invitro-maturation and fertilization, DNA probing and nuclear transfer.

10.1 Objectives

At the end of your study in this unit, you should be able to:

- List the clinical and laboratory process for IVM indications;
- Define DNA probing;
- List some tools and reagents in nuclear transfer;
- Define reprogramming.

10.2 Invitro-Maturation and Fertilization

Invitro- maturation, invitrofertilization and embryo transfer (IVM) is a relatively new assisted reproductive technology (ART). IVM has been used as an alternative choice for ART for polycystic ovary syndrome (PCOS). However, IVM is not the first choice of ART forPCOS patient yet, in spite of less risk of ovarian hyper stimulation syndrome (OHSS).

The reason is not only because of relatively low pregnancy rate, but also poor recognition of IVM in fertility specialists. In Japan, the first IVM pregnancy was achieved in 1999 and pregnancy from frozen thawed embryos derived from IVM in 2000. No known success cases are yet known in Nigeria.

10.3 Clinical and Laboratory Process for IVM Indications

Females with egg factor in which no mature eggs are found (germinal vesicle oocyt only), recurrent fertilization failure, or very poor quality embryos are also IVM targets. Patients who repeatedly failed IVF without distinct causes are IVM candidates, too.

Patient Pretreatment
FSH and hCG administration
FSH is usually administered from day 3 or 5 at dose of 150-300i.u. (FSH primary), the advantage of FSH priming can be described as easier follicular preparation. The efficacy of hCG administration 36 hours prior to that has been reported. Oocytes maturation rate is significantly improved by hCG priming.

The patients require monitoring and oocyte retrieval before further laboratory procedures of culturing.

Nuclear maturation process has been well investigated, but the mechanism of maturation in the cytoplasm remains unclear. Invitro oocyte maturation was observed using electron microscopy. The mitochondria distribution was found to be homogenous in the germinal vesicles, but heterogenous in metaphase I and II. Mitochondria are found to be key factors for oocyte maturation.

10.4 DNA Probing

DNA probing is binding an agent directly to a predefined sequence of nucleic acids. A DNA microarray is a multiple technology used in molecular biology. It consists of an arrayed series of thousands of microscopic spots of DNA oligonucleotides, called features each containing picomoles (10-12 moles) of a specific DNA sequence, known as probes (or reporters) These can be a short section of a gene or other DNA element that are used to hybridize a CDNA or CRNA sample (called target) under high-stringency conditions.

Probe-target hybridization is usually detected and quantified by detection of flourophone-, silver-, or chemiluminescence-labelled targets to determine relative abundance of nucleic acid sequences in the target. Since an array can contain tens of thousands of probes, a microarray experiment can accomplish many genetic tests in parallel. Therefore arrays have dramatically accelerated many types of investigation.

Students' Assessment Exercise 8.1
Question: Define DNA probing.

10.5 Nuclear Transfer

Nuclear transfer is a form of cloning. The steps involves removing the DNA from an oocyte (unfertilized egg), and injecting the nucleus which contains the DNA to be cloned. In rare instances, the newly constructed cell will divide normally, replicating the new DNA while remaining in a pluripotent state.

Despite this, the low efficiency of the technique has prompted some researchers, notably Ian Wilmut, creator of Dolly, the cloned sheep, to abandon it.

10.5.1 Tools and Reagents

Nuclear transfer is a delicate process that is a major hurdle in the development of cloning technology. Materials used in this procedure are a microscope, a holding pipette (small vacuum) to keep the oocyte in place, and a micropipette (hair-thin needle) capable of extracting the nucleus of a cell using a vacuum. For some species, such as mouse, a drill is used to pierce the outer layers of the oocyte.

10.5.2 Somatic Cell Nuclear Transfer

Somatic cell nuclear transfer (SCNT) or therapeutic cloning involves the nucleus of an unfertilized egg cell, replacing it with the material from the nucleus of a "somatic cell" (a skin, heart, or nerve cell, for example), and stimulating this cell to begin dividing. Once the cell begins dividing, stem cells can be extracted 5-6 days later and used for research.

10.5.3 Reprogramming

Genomic reprogramming is the key biological process behind nuclear transfer. Currently, unidentified reprogramming factors present in oocytes are capable of initiating a cascade of events that can reset the mature, specialized cell back to an undifferentiated, embryonic state. These factors are thought to be mainly proteins of the nucleus.

Students' Assessment Exercise 8.2

Question: Define nuclear transfer.

10.6 Conclusion

You have studied the relatively new assisted reproductive technology (ART) of invitro-maturation and fertilization, DNA probing and nuclear transfer. These tools become necessary when there are clinical and laboratory indications of infertility.

10.7 Summary

Invitro-maturation, invitro-fertilization and embryo transfer are relatively new assisted reproductive technologies (ART). DNA probing is binding an agent directly to a predefined sequence of nucleic acids, while nuclear transfer is a form of cloning. These technologies are used when there are medical indications requiring their employment.

Answer to Students' Assessment Exercise 8.1

DNA probing is binding an agent directly to a predefined sequence of nucleic acids.

Answer to Students' Assessment Exercise 8.2

Nuclear Transfer is a form of cloning.

10.8 Tutor-Marked Assignment

Write on the efficiency of Nuclear Transfer.

10.9 References and Further Reading

- Roger Highfield Dolly Creator "Prof" Ian Wilmut Shuns Cloning (hppt://telegraph.co.uk/science.news/3314696/dolly-Creator-Prof-Ian-Wilmut Shuns-cloning,html). The Telegraph, 16 November, 2007.
- http://answers.yahoo.com/question/index?qid=201104055092255AA. Jan 2012

UNIT 11: CLONING

11.0 Introduction

This unit provides you with the basic biological knowledge of the process of cloning. Cloning in biotechnology refers to processes used to create copies of DNA fragments (molecular cloning), cells (cell cloning), or organisms. Your knowledge of this technique will expose you to the limitless possibilities of cloning of animal/plant species.

11.1 Objectives

By the end of your study in this unit, you should be able to:

- Define cloning;
- List examples of species that have been successfully cloned;
- List the steps involved in cloning;
- Discuss the ethical issues of cloning.

11.2 Cloning

In biology, cloning is the process of producing similar populations of genetically identical individuals that occurs in nature when organisms such as bacteria, insects or plants reproduce asexually. Cloning in biotechnology refers to processes used to create copies of DNA fragments (molecular cloning), cells (cell cloning), or organisms. The term also refers to the production of multiple copies of a product such as digital media or software.

The term clone is derived from the Ancient Greek word (klon,"twig"), referring to the process whereby a new plant can be created from a twig. In horticulture, the spelling, clon was used until the twentieth century; the final 'e' came into use to into use to indicate the vowel is a "long O" instead of a "short O". Since the term entered the popular lexicon in a more general context, the spelling, clone, has been used exclusively.

11.3 Molecular Cloning

Molecular cloning refers to the process of making multiple molecules. Cloning is commonly used to amplify DNA fragments containing whole genes, but it can also be used to amplify any DNA sequence such as promoters, non-coding sequences and randomly fragmented DNA. It is used in a wide array of biological experiments and practical applications ranging from genetic finger printing to large scale protein production.

11.4. Steps in Cloning

Cloning of any DNA fragment essentially involves four steps:

1. Fragmentation – breaking apart a strand of DNA
2. Ligation – gluing together pieces of DNA in a desired sequence
3. Transfection – inserting the newly formed pieces of DNA into cells.
4. Screening/Selection – selecting out the cells that were successfully transferred with the new DNA.

Although these steps are invariable among cloning procedures, a number of alternative routes can be selected, these are summarized as a 'cloning strategy'.

Initially, the DNA of interest needs to be isolated to provide a DNA segment of suitable size, subsequently; a ligation procedure is used where the amplified fragment is inserted into a vector (piece of DNA). The vector (which is frequently circular) is linearised using restriction enzymes under appropriate conditions with an enzyme call DNA ligase. Following ligation, the vector with the insert of interest is transfected into cells. A number of alternative techniques are available, such as chemical sensitization of cells, electroporation, optical injection and biolistics. Finally, the transfected cells are cultured. Modern cloning vectors include selectable antibiotic resistance markers, which allow only cells in which the vector has been transfected to grow. Additionally, the cloning vectors may contain colour selection markers, which provide blue/white screening (alpha-factor complementation) on X-gal medium. But this may not necessarily guarantee that the DNA insert is present.

11.5 Cell Cloning

Cloning a cell means to derive a population of cells from a single cell. In the case of unicellular organisms such as bacteria and yeast, this process is remarkably simple and essentially only requires the inoculation of the appropriate medium. However, in the case of cell cultures from multi-cellular organisms, cell cloning is an arduous task as these cells will not readily grow in standard media.

11.6 Cloning Stem Cells

Somatic cells nuclear transfer, known as SCNT, can also be used to create embryos for research or therapeutic purposes. The most likely purpose for this is to produce embryos for use in stem cell research. This process is also called "research cloning" or "therapeutic cloning". The goal is not to create cloned human beings (called "reproductive cloning"), but rather to harvest stem cells that can be used to study human development and to potentially treat diseases. While a clonal human bastocyst has been created, stem cell lines are yet to be isolated from a clonal source.

11.7 Organism Cloning

Organism cloning (also called reproductive cloning) refers to the procedure of creating a new multi-cellular organism, genetically identified to another. In essence, this form of cloning is an asexual method of reproduction, where fertilization or inter-gamete contact does not take place. Asexual reproduction is a naturally occurring phenomenon in many species, including most plants and some insects. Scientists have made some major achievements with cloning, including the asexual reproduction of sheep and cows. There is a lot of ethical debate over whether or not cloning should be used. However, cloning or asexual propagation has been common practice in the horticultural world for hundreds of years.

11.8 Horticultural

Many trees, shrubs, vines, ferus and other herbaceous perennials form clonal colonies naturally. Parts of an individual plant may become detached by fragmentation and grow on to become separate clonal individuals.

11.9 Parthenogenesis

Clonal derivation exists in nature in some animal species and is referred to as parthenogenesis (reproduction of an organism by itself without a male). This is an asexual form of reproduction that is only found in some insects, crustaceans and lizards. The growth and development occurs without fertilization by a male. In plants, parthenogenesis means the development of an embryo from an unfertilized egg cell, and is a component process of apomixis.

In species that use the XY sex-determination system, the offspring will always be female. An example is the "Little Fire Ant" (Wasmanniaauropunctata), which is native to Central and South America, but has spread throughout many tropical environments.

Students' Assessment Exercise 9.1
Question: Define cloning.

11.10 Species Cloned

Dolly the Sheep – Dolly, a Finn-Dorset ewe was the first mammal to have been successfully cloned from an adult cell. Dolly was formed by taking a cell from the udder of her biological mother. Her embryo was created by taking the cell and inserting it into a sheep ovum. The embryo was then placed inside a female sheep that went through a normal pregnancy. She was cloned at the Roslin Institute in Scotland and lived there from her birth in 1996 until her death in 2003 when she was six.

The modern cloning techniques involving nuclear transfer have been successfully performed on several species. Notable experiments include:

11.10.1 Tadpole (1952)

Robert Briggs and Thomas J. King had successfully cloned northern leopard frogs: thirty-five complete embryos and twenty-seven tadpoles from one hundred and four successful nuclear transfers.

11.10.2 Mice (1986)

A mouse was successfully cloned from an early embryonic cell. Soviet Scientists Chaylakhyan, veprencev, Svinidova, and Nikitin had the mouse "masha" cloned. Research was published in the magazine "Biofizika" volume XXXII, issue 5 of 1987.

11.10.3 Cattle: Alpha and Beta (males, 2001) and (2005) Brazil.

11.10.4 Camel (2009): Injaz, is first cloned camel.

11.10.5 Pashmina goat (2012)

Noori, is the first cloned pashmina goat. Scientists at the faculty of veterinary sciences and animal husbandry of sher-e-kashmir University of Agricultural Sciences and Technology of Kashmir successfully cloned the first pashmina goat (Noori) using the advanced reproductive techniques under the leadership of Riaz Ahmad Shah.

11.10.6 Others

Other species cloned include; Carp (1963), Sheep (1984), Rhesus Monkey (2000), Gaur (2001), Cat (2001), Rat (2003), Mule (2003), Horse (2003), Dog (2005), Wolf (2005), Water buffalo (2009) and Pyrenean Ibex (2009).

Students' Assessment Exercise 9.2

Question: List 3 species that have been cloned.

11.11 Human Cloning

Human cloning is the creation of a genetically identical copy of an existing or previously existing human. The term is generally used to refer to artificial human cloning; human clones in the form of identical twins are common place, with their cloning occurring during the natural process of reproduction. There are two commonly discussed types of human cloning; therapeutic cloning and reproductive cloning. Therapeutic cloning involves cloning adult cells for use in medicine and is an active area of research. Reproductive cloning would involve making cloned humans. A third type of cloning called replacement cloning is a theoretical possibility combination between therapeutic. Replacement cloning would entail the replacement of an extensively damaged, failed or failing body through cloning followed by whole or partial brain transplant.

11.12 Ethical Issues of Cloning

Because of recent technological advancements, the cloning of animals (and potentially humans) has been an issue. Many religious organizations oppose all forms of cloning, on the grounds that life begins at conception, Judaism does not equate life with conception and, though some question the wisdom of cloning, Orthodox Judaism rabbis generally find no firm reason in Jewish laws and ethics to object to cloning. Fromthe standpoint of classical liberalism, concerns also exist regarding the protection of the identity of the individual and the right to protect one's genetic identity. Some people may be more open to the idea of cloning of animals because most western countries have passed legislation against cloning humans, yet only a few countries passed legislation against cloning animals.

11.13 Possible Abnormalities Due To Cloning

Researchers have found several abnormalities in cloned organisms, particularly in mice. The cloned organism may be born normal and resemble its non-cloned counterpart, but majority of the time will express changes in its genome later on in life. The concern with cloning humans is that the changes in genomes may not only result in changes in appearance, but in psychological and personality changes as well. The theory behind this is that the biological blue print of the genes is the same in cloned animals as it is in normal ones, but they are read and expressed incorrectly. Results of these abnormally expressed genes in the cloned mice were premature death, pneumonia, liver failure and obesity.

11.14 Cloning Extinct and Endangered Species

Cloning or more precisely, the reconstruction of functional DNA from extinct species has, for decades been a dream of some scientists. Vertebrate cloning poses little risk to the environment, but it can consume scarce conservation resources, and its chances of success in preserving species seem poor. To date, the conservation benefits of transgenics and vertebrate cloning remain entirely theoretical, but many of the risks are known and documented. Conservation biologists should devote their research and energies to the established methods of conservation, none of which require transgenics or vertebrate cloning.

11.15 Conclusion

In this unit cloning has been defined. The steps in cloning were also elaborated, as well as some species of animals that have been successfully cloned. However, there are ethical issues associated with cloning. These have also been discussed in this unit. While cloning is a novel asexual reproductive technology, the ethical issues cannot be ignored.

11.16 Summary

In this unit, cloning, which in biology is the process of producing similar populations of genetically identical individuals that occurs in nature when organisms such as bacteria, insects or plants reproduce asexually, was discussed. There are molecular cloning, cell cloning, cloning stem cells, organism cloning, horticultural cloning and parthenogenesis. Of priority interest to us is the species cloning. Many species have been cloned, but there are ethical issues regarding the cloning that cannot be ignored.

Answers to Students' Assessment Exercise 9.1

Cloning in biotechnology refers to processes used to create copies of DNA fragments or organisms.

Answers to Students' Assessment Exercise 9.2
List of species that have been cloned are:

- Sheep
- Mice
- Cattle

11.17 Tutor-Marked Assignment

a) Define cloning
b) Discuss the steps involved in cloning
c) List some species that have been successfully cloned
d) Discuss your opinion on the ethical issues of cloning

11.18 References

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TMA: ANS 506 TECHNIQUES IN ANIMAL REPRODUCTION

Module 1 (Unit 1)

Question:
How is sex determined in fishes?

Answer:
Most fishes function either as male or female throughout life. i.e. most are bisexual or gonochonistic, as opposed to hermaphroditic, a condition In which an individual produces both eggs and sperm at some stage of its development. Establishment of sex depends on sex chromosomes, designated X and Y in most fishes.

Usually, XX individuals are female and XY individuals are male. There are exceptions, e.g. among the poeciliidea in which the homogametic individual is male. Chromosome designated W and Z are recognized in some of these fishes. Although the sexes have

similar appearances in many species, sexual dimorphism or dichromatism is common in fishes and may be especially well marked in those species with internal fertilization or elaborate reproductive behaviour. Occasional hermaphrodites are found in many gonochoristic species as an abnormality, but numerous species are normally hermaphrodic, with some even capable of self – fertilization.

Module 1 (Unit 2)

Question:
Write notes on heat detection in some farm animals in practice.

Answer:
In sows, intact boar can be introduced into a pen of females and observation made of those females the boar attempts to mount. Back pressure can also be applied on the suspected sow on heat and she could be immobile if on heat.

In ewes, vasectomized rams can be fitted with marking crayons. The raddled ram can be made to run with the ewe flock. Ewes on heat will stand to be mounted and marked.

In the cow, intact or vasectomized bull can be fitted with a chin-ball marker and introduced into a herd of cows. Cows on heat will stand to be mounted and marked.

Module 2 (Unit 3)

Question:
Elaborate on the concept of cryopreservation.

Answer:
Cryopreservation is a process where cells or whole tissues are preserved by cooling to sub – zero temperature, typically – 196^{o}C, the boiling point of liquid nitrogen. If cryoprotectant solutions are not used, the cells being preserved are likely to be damaged due to freezing during the cooling or thawing process.

Generally, cryopreservation is easier for thin samples and small clumps of individual cells, because these can be cooled more quickly and so require lesser doses of toxic cryoprotectants.

Cryopreservation has been done for;

- Semen.
- Blood, special cells for transfusion, stem cells, umbilical cord blood.
- Tissue samples like tumors and histological cross sections.
- Eggs (oocytes).
- Embryos that are 2, 4 or 8 cells
- Ovarian tissue.
- Plant seeds or shoots.

Module 2 (Unit 4)

Question:

Discuss the factors affecting fertility during Artificial Insemination.

Answer:
Factors affecting fertility during artificial insemination include;

1. Method of semen collection or initial semen quality;
2. Species, breed and individual differences;
3. Semen preservation method;
4. Processing method;

- Holding time before process.
- Choice of diluents.
- Dilution temperature – should be at about $30^{o}C$.
- Dilution ratio, (i-4, semen: diluents) best.
- Method of dilution (Mix gradually).
- Diluents composition.
- Cooling time to $50^{o}C$.
- Freezing Method e.g. Pellet freezing on dry ice, straw freezing in liquid nitrogen vapour.
- Storage temperature – the lower the better .
- Length of storage, the smaller the better.
- Thawing temperature (should be $37^{o}C$).
- Post thawing incubation period, the longer the poorer. Use immediately.

5. Timing of insemination;
6. Insemination dose;
7. Skill of the technician.

Module 3 (Unit 5)

Question:
Define oestrus synchronization and discuss any advantages.

Answer:
Oestrus synchronization is a term used to indicate the process of bringing groups of animals into heat together in response to some form of treatment. Such animals should therefore conceive at closely similar times, proceed through pregnancy together and produce their offsprings in or within a short period.

Advantages;

a) It enables regulation of time of heat and possibly ovulation.
b) Enables uniform group feeding, supervision, cross fostering, batch weaning, fattening and marketing.
c) Enables rationalization of the use of labour, buildings and other resources.

Module 3 (Unit 6)

Question:

Describe the process of embryo transplantation.

Answer:
The process requires recovery of embryos be flushing fluid through the reproductive tract of the donor (which may or may not have been super ovulated), examination of the embryos under a binocular microscope and then their insertion into the reproductive tract of the recipient or foster mother using a glass pipette.

The recovery and transplantation procedure may involve abnormal surgery under full or local anesthesia, or they may gain access to the uterus of conscious animals through the vagina and cervix as in artificial insemination.

Module 4 (Unit 7)

Question:
Describe any two laboratory tests for pregnancy diagnosis in cows.

Answer:
The various laboratory tests developed for pregnancy diagnosis in domestic animals are indirect methods of pregnancy evaluation and utilize qualitative or quantitative measures of reproductive hormones at specific stages after artificial insemination or mating or detect conceptions specific substances in maternal body parts or body fluid as indirect indicators of the presence of a viable pregnancy. Two of these tests in cows are:

I. Progesterone hormone assay:- The corpus luteum formed on the ovary subsequent to ovulation produces progesterone for maintenance of pregnancy for a reasonable period in some species and for entire gestation in the species like the cow, buffalo, goat and sow. The elevated progesterone levels are used as means of pregnancy diagnosis.
II. Estrone sulfate:- The estrone sulfate is produced by the fetomaternal axis or the conceptus and therefore its presence in urine, milk, feces or blood is an indicator of pregnancy. The detection of these hormones depends on availability of appropriate kits and personnel. Others are:
    - Cuboni test.
    - The mouse test.
    - Assay of Gonadotiopins.
    - Biologic tests e.g. AscheimZondek test, the Friedman test (rabbit test) or the frog or toad test. Etc

Module 4 (Unit 8)

Answer to TMA 8.10

Question:
Elaborate on some induction of parturition indicators.

Answer:

Induction of Parturition should only be embarked upon as a last resort. Induction of parturition indicators include;

I. Managemental causes as you desire that animal should participate in competition.
II. Health conditions. Where health of dam is deteriorating. If induction is done two weeks to date of normal birth, survivability is better.
III. To avoid dystocia because during enhanced and daily weight gain of fetus is increased.
IV. To avoid udder injuries or excessive udder edema.
V. Truncate the calving season, thereby allowing more time post partum to resume cyclicity before the next breeding season.

Question:
Elaborate on the concept of induction of parturition.

Answer:
The concept of induction of parturition has to do with the bringing out of parturition. Parturition is the process of bringing forth the young by the dam. The process of giving birth. Hormones are used to induce parturition in animals. Estrogen is used in various induction regimes in an attempt to mimic normal hormonal events at parturition, followed by oxytocin and or PGF2x. Estrogen in conjunction with dexamethasone were used to mimic parturition in beef cattle, with some fair success.

Module 5 (Unit 9)

Question:
Write short notes on any three (3) methods of weaning.

Answer:
Circumstances on the farm determine the method of weaning. The following methods can be used.

1. Keep the calves in a kraal or well – fenced camp and remove the cows to a distant camp, preferably out of earshot of the calves.

2. Remove the cows temporally from a camp and in their absence move the calves to anther distant camp. Cows tend to look for their calves in the camp in which they were last seen and this method should prevent the cows from breaking out of the camp.

3. Exchange calves from two different herds. The calves will then have the company of cows. Some cross – sucking is however, likely to occur.

4. Separate the cows and calves by a strong close – strand wire fence. This method can reduce weaning stress.

5. Nose plates, commercially available or home – made can be fitted to calves for 7 to 4 days. These prevent sucking, even if cows and calves remain

together throughout the weaning period. When the nose plates are removed, the cows and calves are separated, but with relatively little stress.

Module 5(Unit 10)

Question:
Write on the efficiency of Nuclear Transfer.

Answer:
Nuclear transfer is a form of cloning. The steps involves removing the DNA from an oocyte (unfertilized egg), and injecting the nucleus which contains the DNA to be cloned. In rare instances, the newly constructed cell will divide normally, replicating the new DNA while remaining in a pluripotent state. Despite this, the efficiency of the technique is low.

Module 6 (Unit 11)

Question:
Attempt any three (3) out of the four (4) questions below.

a) Define cloning.
b) Discuss the steps in cloning.
c) List some species that have been successfully cloned.
d) Discuss your opinion on the ethical issues of cloning.

Answer:
Cloning is the process of producing similar population of genetically identical individuals.

a) Steps in cloning include the following:
I. Fragmentation – breaking apart a straud of DNA;
II. Ligation – gluing together pieces of DNA in a desired sequence;
III. Transfection – inserting the newly formed pieces of DNA into cells;
IV. Screening/selection – selecting out the cells that were successfully transferred with the new DNA.

Although these steps are invariable among cloning procedures, a number of alternative routes can be selected, these are summarized as a ‘cloning strategy’.

b) Listed below is some species that have been successfully cloned;
I. Dolly the sheep
II. Tadpole
III. Mice
IV. Cattle
V. Camel
VI. Pashmina goat
VII. Carp
VIII. Others are Rhesus Monkey, Cat, Dog mule etc

c) My opinion on the ethical issues of cloning.
As a religious person, I oppose all forms of cloning on the grounds that life out ought to begin at conception, not another organism having its kind identically reproduced.

GLOSSARY OF TERMS

Abdominal Ballottement – This involves palpation to determine pregnancy by pushing up against the uterus with the finger so as to feel any embryo as it sinks back in to place in the amniotic fluid.

Artificial Insemination (AI) – The possible impregnation of a female by artificial introduction of semen taken from a male.

Cloning – The process used to create copies of DNA fragments (molecular cloning), cells (cell cloning), or organisms. It is the process of producing similar populations of genetically identical individuals.

Oestrus (Heat) – The time within the oestrous cycle when the female animal stands to be mounted and mated by the male.

Cryopreservation – A process where cells or whole tissues are preserved by cooling to sub – zero temperature typically – 196^{o}C.

DNA Probing – The binding of an agent directly to a predefined sequence of nucleic acids.

Embryo Transfer (ET) – The technique of recovering embryos from the reproductive tract of the donor animal and then inserting same into the reproductive tract of the recipient or foster mother.

Non Return to Oestrus – When an animal is mated or inseminated and it does not return to oestrus.

Nuclear Transfer – This is a form of cloning.

Oestrous Cycle – The cycle from one period of heat to the other in the same female animal.

Sex – The sum total of those differences in structure and function on the basis of which an organism is classified as male or female.

Super Ovulation – Used when in response to some treatment, the number of follicles ovulating is significantly above the normal ovulation rate for the species or breed in question.

Synchronization of Oestrus – a term used to indicate the process of bringing groups of animals into heat together in response to some form of treatment.

Time of Ovulation – The moment of follicular collapse with release of egg(s).

Weaning – The process of causing the young to become accustomed gradually to food other than its mother's milk.