Department of Animal Nutrition, Genetics & Breeding
Course Title: Reproduction of Farm Animals & Biotechnology
Course Code: ANGB 455
Level-4, Semester-II
Credit Hours: 3, Contact Hour: 3

Course Content (Theory)

1. **Introduction:** Definition and scope of Animal Reproduction; Type of reproduction in various species of animals, Relationship with genetics and breeding.

2. **Reproductive Hormone:** Hormones and receptor, Classification, Properties, function and mode of action; Hormonal control of male and female reproduction, Hormone assay**,** Hormone like substances-growth factors and prostaglandins, Interaction between genetics and endocrinology.

3. **Reproductive cycle and sexual behavior:** Puberty and its practical application, Estrous cycle and related events in different ruminants, Breeding season and its effect on reproduction, Maternal and neonatal behavior.

4. **Fertilization:** Fertilization and preparation of gametes.

5. **Herd fertility:** Concept of fertility and sterility, Reproductive failure and measures of reproductive efficiency in male and female.

6. **Artificial Insemination:** Recent advances, advantages and limitations of artificial insemination; Semen physiology and sperm biology, Composition and properties of semen, structure, physiology and fertilizing ability of sperm, Survival of sperm *in vivo* and *in vitro*; Semen evaluation, diluents and their composition; Management of females, preparation and examination of AI bulls; Management of breeding males, teasers; Insemination techniques, time and fertility, maintenance of AI records.

7. **Reproductive Biotechnology:** Concept, scope and its application in animal industry; Background of Hormonal Estrus synchronization; Multiple ovulation and Embryo Transfer (MOET); *In vitro* maturation (IVM) and *in vitro* fertilization (IVF) of mammalian oocyte, *in vitro* culture (IVC) of embryos and their transfer in surrogate mother; Frozen semen production technology; Cryopreservation of semen, oocyte and embryos; Principal of cloning, Methods and application of cloning in animal; Embryo cloning: Concepts and consequences, embryo slicing and their application in modern animal production; Embryonic stem cells culture; Transgenic animals; Knock-out mouse.

Chapter 1
Introduction

Reproduction: Reproduction is the process of production of new individuals those are more or less similar to itself. It is a complex science. In order to understand the science of reproduction, it is necessary to include anatomy, physiology, endocrinology, embryology, histology, cytology, microbiology and some nutrition.

Reproduction involves a science of physiological and psychological events that must be properly timed. The endocrine system, through a production of several hormones is responsible for this timing.

- Reproduction is the process by which animals produce offspring for the purpose of continuing the species.
- The process of reproduction begins with copulation, which is the mating of a male and female of the species.
- Sperm cells from the male are deposited in the female reproductive tract and try to unite with an egg cell.

When fertilization (a sperm cell and egg cell unite) occurs, an embryo begins to develop.

Objective of the study of reproduction:

- To understand the physiology of reproduction of farm animals.
- To get acquainting with the modern techniques so far for the improvement of reproductive efficiency of animals.
- To control the reproductive system of animals.
- To produce more economically viable offspring from the animal.

Purpose of reproduction:

Reproduction has at least three purposes:

1. Perpetuation of the species
2. To provide food
3. Genetic improvement

Reproduction strategies:

Both asexual and sexual reproduction occurs in the animal kingdom:

- Sexual reproduction is creation of offspring by fusion of male gametes (sperm) and female gametes (eggs) to form zygotes.
- External fertilization; fish etc.
- Internal fertilization a) external incubation; birds etc. b) internal incubation-mammals etc.

- Asexual reproduction is creation of offspring whose genes all come from one parent

- In budding two new individuals arise from outgrowths of existing ones
- Fragmentation is breaking of the body into pieces, some or all of which develop into adults
- Parthenogenesis is a process in which an egg develops without being fertilized. Several genera of fishes, amphibians and lizard reproduce only by a complex form of parthenogenesis (budding, spore formation).

Cloning, nuclear transfer etc. in higher animals and bacteria algae yeast as lower organisms.

Reproductive differences in species:

- Sexual seasons
- Gestation periods
- Types of placentation
- Litter size
- Lactation period
- Susceptibility to reproductive diseases.

Factors responsible for reproduction:

- Seasons of the year
- Length of the heat period
- Duration of heat period
- Gestation period
- Sexual activity with the life time
- Parental litter size
- Susceptibility to reproductive disease
- Nutrition

Classification of animal according to the reproductive behavior

Types	Character	Examples
Oviparous	Lay large number of eggs, abundant yolk and hatched outside the body, external genitalia are poorly developed	Birds, chickens, fishes, amphibians
Ovoviviparous	Their eggs are covered by protective shell, large amount of yolk and larvae hatch inside the body of the female	Reptiles
Viviparous	Fewer number of eggs with scant yolk fertilization is internal fetus developed in	mammals

	uterus and external genitalia are well developed	

Role of male:

- Produce mature spermatozoa
- Store spermatozoa until needed
- Discharge spermatozoa in a convenient fluid into the female so that they may ascend the female tract and fertilize the egg
- Provide the proper behavior pattern (courtship)

Role of female:

- Provide egg (ova)
- Provide proper environment for fertilization
- Nurture the embryo/ fetus
- Deliver immature individual for exterior
- Lactation
- Provide for proper behavior pattern
    i. Attracting male and female
    ii. Maternal function

Relationship between Animal Genetics and Animal Breeding with Animal Reproduction:

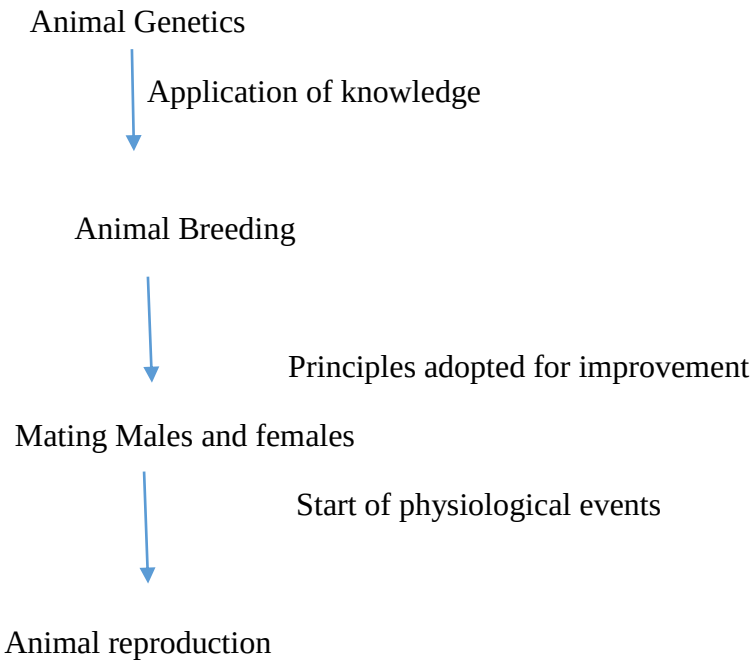


Fig: Relationship between animal genetics and animal breeding with animal reproduction

Scope of animal reproduction

The scope of animal reproduction can be described as the following two ways

- **Traditional scope:** It deals with the application of knowledge and principle of the animal genetics and breeding to which rapid improvement of animal is possible.
    1. Orientation with reproductive system
    2. Endocrinology of reproduction
    3. Puberty, reproductive cycle, sexual behavior
    4. Estrous detection
    5. Artificial insemination
    6. Pregnancy diagnosis and parturition
    7. Reproductive efficiency, failure and diseases
- **Modern scope:** Reproductive biotechnology is the modern tool of reproduction which includes assisted reproductive biotechnologies are
    1. Artificial insemination
    2. In vitro development of small follicle
    3. In vitro maturation of oocytes
    4. In vitro fertilization and culture of egg
    5. Oocytes and embryo preservation
    6. Embryo transfer technologies
    7. Genetic engineering and cloning
    8. Seperation of X and Y spermatozoa
    9. Embryo sexing
    10. Ovum pic-up (OPU)
    11. Production of transgenic animal and other such like activites.

Reproductive system and function

Reproductive system is hanging through broad ligaments-

- Mesovarium = ovary
- Mesosalpinx = oviduct
- Mesometrium = uterus

Female reproductive organ consists of-

1. Primary organ = ovary (2)
2. Secondary organ =
    a. Oviduct/ fallopian tube or uterine tube (infundibulum, ampulla, isthmus)

b. Uterus
    i. Horn of the uterus = 2
    ii. Body of the uterus = 1

c. Cervix
d. Vagina
e. Vestibule
f. Vulva

Function:

Ovary

Exocrine = to release egg/ ovum/ gamete

Endocrine: to secrete hormone

- Estrogen (to manifest femaleness)
- Progesterone (pregnancy maintain)

Oviduct : (infundibulum, ampulla, isthmus)

i. To receive ova released from ovary
ii. To carry ova upto fertilization take place
iii. To carry sperm upto the fertilization take place
iv. To carry zygote to the horn of uterus
v. To create fertlization environment through oviductal fluid

Uterus:

i. To carry the sperm from the body of uterus to oviduct
ii. Regukation of the function of the CL
iii. Initiation of implantation, pregnancy and parturition
iv. Site of development of zygote to fetus

Cervix:

i. It keeps strong barrier between the external and internal genitialia
ii. It can select the quality or active sperm

Vagina: (cow-25-30cm, ewe-10-14cm, pig-10-15 cm, mare-20-35cm)

i. It is copulatory organ

ii. It act as birth canal
iii. It act as urinary canal

Vulva: it is the common opening for female genital system and urinary system

Accessory reproductive/ sex gland of female are- mammary gland/ udder.

Male reproductive organ consists of-

1. Primary organ= testis (sperm and hormone produce)
2. Secondary organ-
    i. Epididymis= store of the sperm and maturity of sperm
    ii. Ductus deference = to carry the sperm from epididymis to urethra (from tail of epididymis to urethra region)
    iii. Urethra = common opening of urinary bladder plus vas diffference and accessory gland etc.
    iv. Penis (root of penis, body of penis and glans penis)

Accessory gland = prostedgland, bulbo urethral gland, seminal vesicle)

Embryology of the female reproductive system

It consists of

1. Two undifferentiated gonads
2. Two pairs of ducts
3. A urogenital sinus
4. A genital tubercle
5. Vestibular folds

Sex of the foetus depends on-

- Inherited genes
- Gonadogenesis
- Formation and maturation of accessory reproductive organ

Table : Developmental fate of the sexual rudiments in the male and female mammalian fetus

Sexual rudiment	Male	Female
Gonad • Cortex • Medulla	 Regress Testis	 Ovary Regress
Mullerian ducts	Vestiges	Uterus, oviducts, parts of vagina
Wolffian ducts	Urethra, prostate,bulbourethral glands	Parts of vagina, urethra
Genital tubercle (phallus)	Penis	Clitoris
Vestibular folds	Scrotum	Labia

Chapter 2
Reproductive Hormone

- Hormone
- Receptor
- Endocrinology of reproduction
- Biochemical characteristics of hormone
- General effects of hormone
- Classification of hormone
- Organizing of control system of hormone governing male and female reproduction

Hormone: Any susbtance formed in very small amount in one specialized organ or group of cells and carried to another organ or group of cells, in the same organisms, upon which it has a specific regulatory action. i.e. FSH, LH, estrogen, progesterone, testosteron etc.

Receptor: Any cellular macromolecules that undergo combination with a hormone, neurotransmitter, drug or intracellular messenger to initiate a change in cell function. Receptors are concerned directly and specially in chemical signaling between and within cells. Intracellular receptors e.g. Steroid-hormone receptor, bind ligands that enter the cell across the plasma membrane.

Or, Hormone receptors are binding sites on the target cell (either on the surface or in the cytoplasm or nucleus of the target cell) that are activated only when specific hormones bind to them. If a hormones does not/ can not bind to its receptor, then no physiologic effects results.

Endocrinology of reproduction: It is deals with biochemistry, physiology, pharmacology, molecular biology of hormone and their receptors. Hormone sysnthesized and secreted by endocrine glands are transported into the blood circulatory system to stimulate, inhibit or interact with the functional activity or specific target organ producing a wide range of physiological response.

Biochemical characteristics of hormone

- Don’t supply energy to reaction
- It acts in minute amount
- Rapidly remove from the blood stream
- It regulates the rate of reaction but don’t initiate any reaction

General effects of hormone

- Morphologenesis
- Maintenance the internal environment
- Integrates the physilogical events

Characteristics of receptor

Hormone receptors are proteins or glycoproteins that are able to function as follows:

- They distinguish their hormone from other molecules that may have very similar stucture
- They bind to the hormone (sometimes called a ligand) even when its concentration is exceedingly low.
- They undergo a conformational change when bound to the hormone.
- They catalyze biochemical events or transmit changes in molecular conformation to adjanacent molecules, producing a biochemical changes.

Classification of hormones:

According to type of action:

a) **Primary hormone**: are those that are directly involved in various aspects of reproduction, sexual behavior, spermatogenesis, ovulation, fertilization, implantation, maintenance of gestation, parturition, lactation and maternal behavior. Eg. FSH, Testosterone, Progesterone, Estrogen etc.
b) **Secondary hormone:** are those that indirectly involve in various aspects of reproduction, they influnce growth, development and metabolism. Eg. Somatotropin hormone (growth hormone).

According to chemical structure: the primary hormones of reproduction, based on their chemical structure are divided into three groups:

Hormones	Molecular wt (dalton)	Characteristics
Steroid	300-400	Natural steroid are not effective by oral administration, synthetic and plant steroid can be administrated orally or by injection, eg. Estrogen, Progesterone, Vit-D.
Protein	300-70000	Easily broken down by enzymes, can not be given orally, must be administrated by injection. E.g. LH, insulin, hCG, FSH
Fatty acid	400	Can be administered only by injection.

According to the source of origin

1. Gonadal hormone, male and female hormone
2. Placental hormone, hCG
3. Pituitary hormone. FSH,LH, Oxytocin

4. Hypothalamic hormone, FSH-RH, LH-RH, Gn-RH

Table: Techniques used in reproductive endocrinology

Procedure	Protocol and application
Ablation gland	Surgical revomal of endocrine gland leads to a deficiency of hormone produced by that gland. Castration of males removes androgen, resulting in a change in a physical characteristics.
Replacement therapy	Deficiency caused by ablation may be overcome by implantation of the gland back into the animal or by injection of crude extracts from remove gland
Isolation of hormone	Isolation or separation of hormone from other substances in the crude extract of endocrine gland involves intensive chemical separation procedures coupled with sensitive assays to measure the hormone Once hormone is isolated, it is chemically identified and synthesized if possible.
Regulation of endocrine gland	Change in synthesis and release rate of hormone from endocrine gland studied under endocrine physiological condition by assay techniques

Chapter 3
Reproductive cycle and sexual behavior

Puberty

Puberty can be defined as the age of the female at which estrus is the first detected and is followed by characteristics cyclic ovarian activity in the non-pregnant. Perry (1971) has suggested that puberty be viewed as a gradual and quantitative phenomenon rather than as a sudden and qualitative endocrinological event.

Donovan et al. (1965) regarded puberty as the entire period during which the gonads secrets steroids in amounts sufficient to cause accelerated growth of the genital organs and the appearance of secondary sexual characteristics. Thus puberty is likely to remain the age at which animals are competent to reproduce successfully. A male oe female animal has reached puberty when it is able to release gametes and to manifest complete sexual behavior sequence. Puberty is basically the results of gradual adjustment between increasing gonadotropic activity and the ability of gonad to simultaneously assume steroidogenesis and gametogenesis.

Table: Some common observed measurements of the age at puberty, together with characteristics of the estrous cycle in large farm species.

Characteristics	Cow	Sheep	Doe	Pig	Horse	Rabbit
Age of puberty (Months)	9-13	5-10	8-9	5-7	15-24	3-4
Length of estrous cycle (Days)	20-21	16-17	15-24	20-22	21-22	
Length of luteal phase(Days)	17-18	14-15	18-19	15-16	14-15	
Duration of estrous(Hours)	12-26	24-36	36-48	40-70	96-168 (4-5 days)	
Timing of ovulation(hrs)	10-12 after ends of oestrus	24-26 after onset of estrus	30-36 after onset of estrus	36-40 after onset of estrus	24-36 before end of estrus	
Time of AI	12-18 hrs after onset of estrus	12-18 hrs after onset of estrus	12-18 hrs after onset of estrus	16-24 hrs after onset of estrus	Second day or everyday	

Age of puberty is influenced by-

- Physical environment
- Photoperiod
- Age and breed of dam
- Breed of sire
- Sires within breed
- Heterosis
- Environmental temperature
- Body weight as affected by nutrition
- Growth rate before and after weaning

Practical application of age of puberty

The age of sexual maturity in ewes is related to adequate energy intake and the attainment of sufficient body weight. Early onset of sexual maturity provides economic advantages through increase lifetime reproductive tract. Thus, it is advantageous to maximize growth rates in ewe lambs being added to the breeding flock. The genetic improvement achieved by artificial insemination of dairy cattle has resulted from the use of proven tested sires. Obtaining semen at the earliest possible age from bulls being proven tested is desirable to hasten identification of superior sires.

Estrous cycle

Phases of the estrous cycle

The basic pattern of the estrous cycle is the same in all domestic animals, but some specific differences are found in specific parts of the cycle. Some specifics about relevant farm species are summarized in Table 13.

Table 13. *Average ages or times of reproductive variables selected by species*

Animal	*Onset of Puberty*	*Age of first Fervice*	*Estrus Cycle*	*Estrus*	*Gestation*
Mare	18 mo	2-3 yr	21 d	6 d	336 d
Cow	1-2 yr	1-2 yr	21 d	18 h	282 d
Ewe	8 mo	1-1.5yr	17 d	1-2 d	150 d
Sow	7 mo	8-10 mo	21 d	2 d	114 d

The estrous cycle (Fig. 12.2.) may be divided into several phases based on behaviour changes or structural changes in internal and external genitalia.

Proestrus

The first phase (proestrus) of the estrous cycle is the building-up phase. During this phase the ovarian follicle (under the influence of FSH and LH) enlarges and begins to secrete estrogens. In polyestrous species, proestrus usually begins within a day or two of regression of the corpus luteum from the previous cycle. Estrogenes absorbed from the follicles into the blood stimulate increases in vascularity and cell growth of the tubular genitalia in preparation for estrus and pregnancy. Late in proestrus the vaginal wall thickness, and the external genitalia may increase in vascularity (e.g., swelling, and redness) in preparation for copulation. In some species, the vulva may discharge mucus late in proestrus.

Estrus

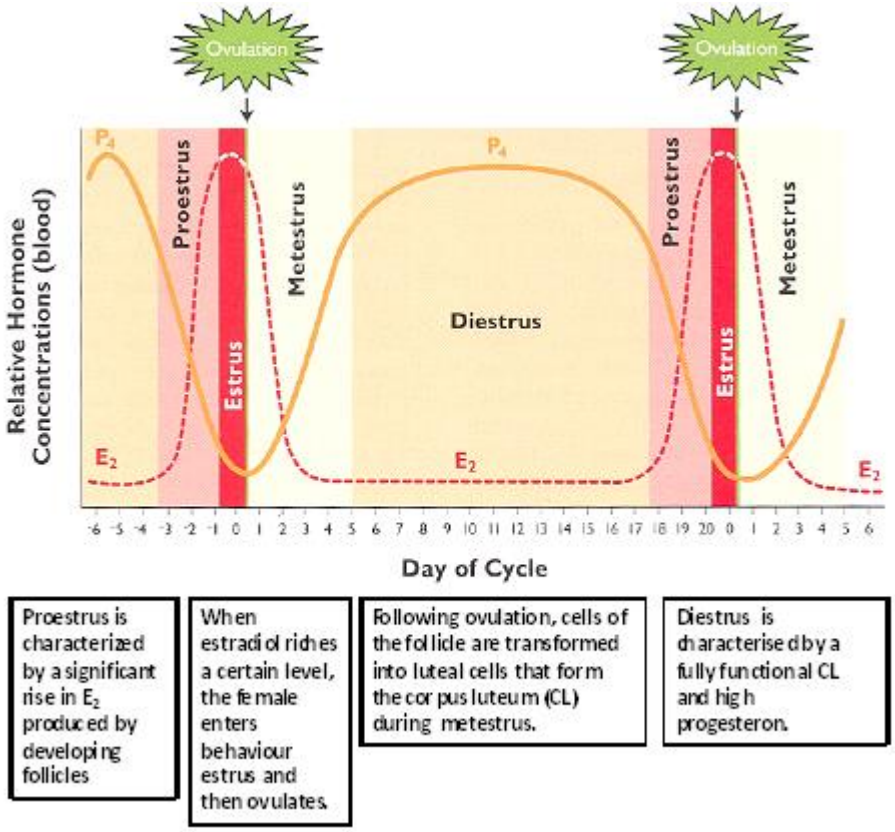
Estrus , the period of sexual receptivity, is primarily initiated by the elevation in estrogens from mature follicles just prior to ovulation. In most domestic species, ovulation occurs within a day or two after the onset of behavioural estrus, which is about the end of behavioural estrus. Progesterone from preovulatory follicles, developing corpora lutea, or corpora lutea from previous cycles also promotes behaviours estrus in some species.

Proestrus and estrus together comprise the follicular phase of the reproductive cycle.

Metestrus

The end of sexual receptivity marks the beginning of metestrus, the postovulatory phase dominated by corpus luteum function. During this period, serum estrogens decrease and progesterone increases. A fully developed corpus luteum has a notable influence on the uterus.

Figure 12.2. Fig 12.2.: Stages of estrous cycle (E2: estradiol); Senger (2003)



The endometrial lining of the uterus thickens; uterine glands enlarge; and uterine muscles show increased development. The external genitalia return to their state before estrus as plasma estrogens decrease.

Diestrus and anestrus

Polyestrous animals have a short period of inactivity before the proestrus phase of the next cycle. This is diestrus. Animals with long periods between cycles or polyestrous animals that stop cycling (e.g., due to change in season) enter a long period of inactivity termed anestrus. For example, sheep have a short diestrus while cycling during a breeding season but enter anestrus if pregnancy is not established during the breeding season. During anestrus the uterine tubes, and vagina shrink, and remain small until the next breeding season.

Puberty

Puberty in female animals can be defined as the first estrus accompanied by ovulation. The endocrine basis for puberty in females is the development of the hypothalamic mechanisms responsible for GnRH release. The adenohypophysis is capable of releasing FSH and LH before

GnRH becomes available to stimulate their release. Great variations in the timing of puberty can be found within a single species, depending on climate, level of nutrition, and heredity.

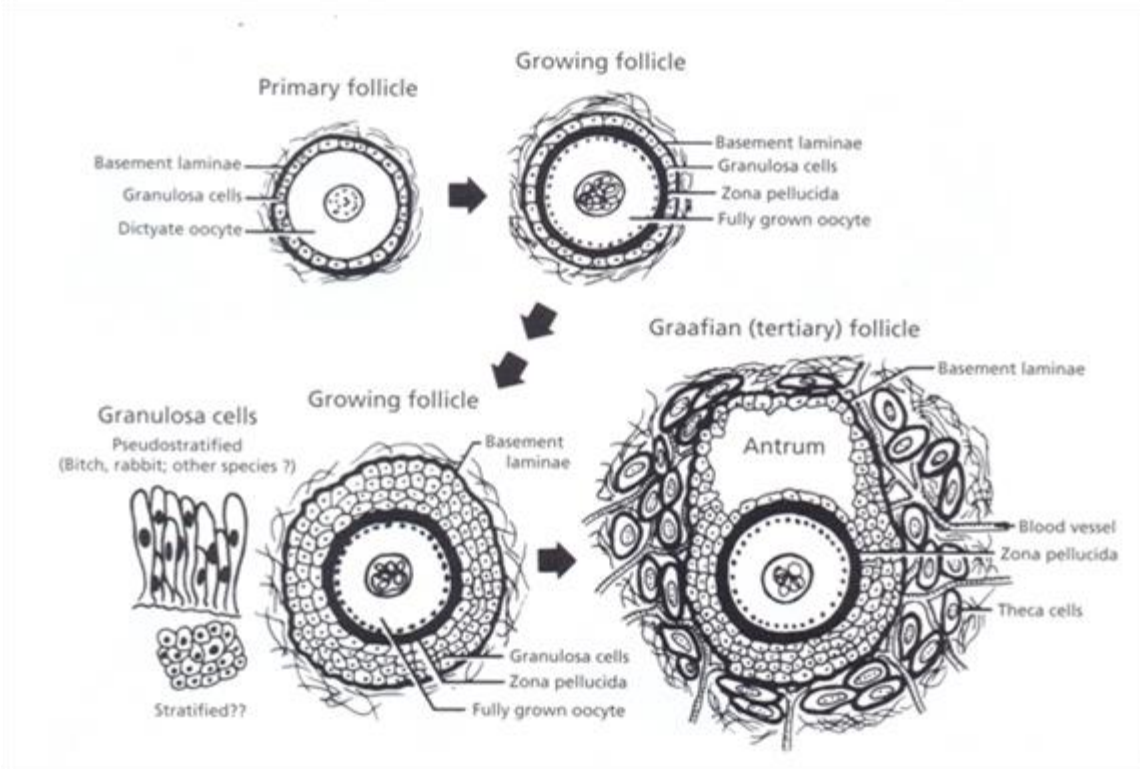
OOGENESIS

In the fetus, primordial germ cells migrate from the yolk sac to the developing ovaries, where a single layer of follicular cells surrounds a germ cell destined to become an ovum. The central germ cell (now termed an oogonium) enlarges and begins meiosis. (Recall that meiosis entails two cell divisions during which the diploid number of chromosomes is reduced by half to the haploid number.) The oogonium does not complete meiosis; it stops in the first prophase before the first division. At this stage, the developing ovum is a primary oocyt, and the combination of a primary oocyte and its surrounding cuboidal follicular cell (granulos cell) layer is a primary follicle (Fig.12. 3). At birth, the ovaries of most domestic species contain hundreds of thousands of primary follicles waiting to continue their development. What determines which of the thousands of primary follicles is selected to develop further during a specific estrous cycle is unknown. In contrast to spermatogenesis, which produces four spermatozoa from each primary germ cells, the maturation of the primary oocyte results in only one mature ovum and three rudimentary cells, called polar bodies. In most animals, the first of the two meiotic divisions is complete, resulting in the formation of the first polar body, before or immediately after ovulation (the discharge of an oocyte from a follicle).

Secondary follicles

In all animals, multiple primary follicles typically begin further development during a single estrous cycle. In monotocous animals (animals not bearing litters and normally having only one offspring per gestation, such as the mare and cow), one follicle usually develops more

Figure 12.3. Figure 12.3: Development of an ovarian follicle from its primordial form to a Graafian follicle (Reece, 2009)

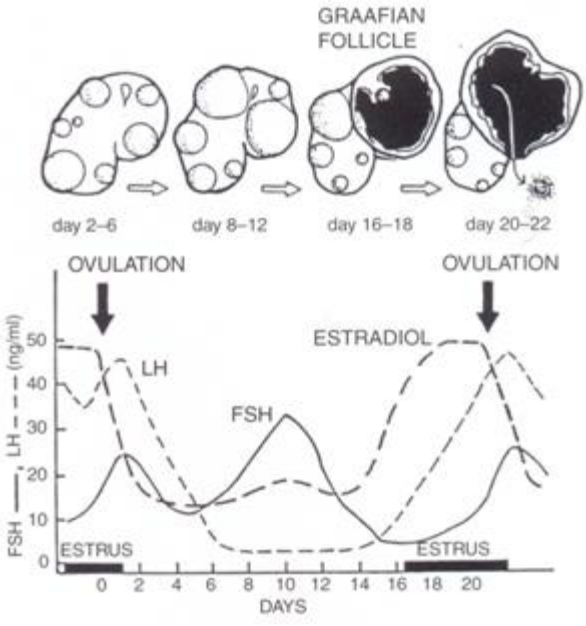


rapidly than others, and only one ovum is released at ovulation. The rest of the developing follicles regress and form atretic follicles. Polytocous animals, such as carnivores and swine, which normally produce two or more offspring per gestation, usually have several follicles that develop and ovulate at approximately the same time. The ova may all come from one ovary, or some may come from each ovary.

The further development of primary follicles includes enlargement of the oocyte and replication of the surrounding follicular cells. The replicating follicular cells become several layers thick, and this surrounding group of cells is a granulosa. The granulose cells secrete glycoproteins that cross-link to form a protective shell, the zona pellucid, around the oocyte (Fig. 12.3). cytoplasmic process processes of granulose cells penetrate the zona to permit communication and exchange between them and the oocyte. The initial development to this point is independent of hormonal stimulation by gonadotrophins (follicle-stimulating hormone [FSH] and luteinizing hormone [LH]; Fig. 12.4.).

The developing follicle is termed a secondary follicle when the oocyte has enlarged and is surrounded by a developing granulose. A theca, consisting of layers of cells immediately surrounding the granulosa, also first develops late during the secondary follicle stage.

Figure 12.4. Figure 12.4.: Hormone patterns during estrus in mare McKinnon et al. (2011)



Breeding season

All female have the tendency to show seasonal cyclic changes in ovarian activity, but it is the degree of ovarian activity or inactivity which influenced by the environment. Restricted breeding seasons are the more natural state of all animals except those have natural habitate in the the tropics. Loss of breeding season tendencies is really a response of the animal to its association with man.

There are some factors which elicit the seasonal response:

- Effect of loght
- Effect of temperature
- Effect of feed supply
- Effect of psychological factors

Ram can mate throughout the year. But testis weight, testosterone and gonadotropins level minimum in (Jan-may). During female and estrus in cattle and pig, estrous occurs regularly throughout the year. Local breeding condition often must its expression.

Role of photo-periodism and temperature

The photo-periodism and environmental temperature after the annual sexual cycle. However, photoperiodism affects much more than temperature. When ewes are placed under 12hrs of day light every day or under constant illumination for many year, a breeding season is maintained for 1 or 2 year. Then estrus become more random throughout the year. Photoperiodism, temperature and other environmental factor affect reproductive physiology through endocrine and neuroendocrine mechanism.

Its effect on reproduction

It is obvious that there are great variations of reproduction of animals. Reproduction patterns of animals in their natural environments differ greatly from those of highly domesticated animals which have become accustomed to a protected environment. The reproductive patterns of the animals under natural conditions, far different from domestic environment, tends toward a patterns by which the young are delivered at this time of the year when temperature and feed availability are optimum.

Pheromones

Pheromones are chemical substances produces on the exterior surface of animals. These chemical messengers do not meet the definition of hormone but must be recognized as members of the broader concept of a hormone or external chemical messenger, these odoriferous substances serve as communication media between animals. Some of the pheromones are used to communicate information concerned with reproduction and they are termed as sex pheromones. The usual source of sex pheromones are modified skin glands.

Chapter 4
Fertilization

Fertilization: Penetration of the ovum by a spermatozoon and completed by the fusion of the female and male chromosomes with subsequent formation of male and female pronuclei and expulsion of the second polar body, syngamy leading to cleavage to two blastomeres.

Fertilization is the fusion of a female's egg cell (oocyte) and a male's sperm cell (spermatozoa) to form the first cell of a new and unique being. White on the surface this sounds like simple process, there are many factors that make this possible.

Events:

1. Gamete maturation (ovum and sperm maturation)
2. Sperm-oocyte encounter
3. Cumulus penetration
4. Sperm attachment
5. Zona penetration
6. Gamete fusion
7. Block to polyspermy
8. Development of pronuclei
9. Syngamy

Sperm and egg cell are not only different from other cells, but are different from each other. A female is born with all the eggs she will ever have. At birth, the chromosomes of these eggs have only completed the beginning of meiosis (meiosis I) and will remain dormant (inactive) until onset of estrus cycle in puberty. Meiosis is suspended for the second time in the middle of Meiosis II around just prior to ovulation and does not resumes unless fertilization occurs.

1. **Ovum maturation:** ovum resumes the process of meiosis from prophase I of the first meiotic division. In most species the ovum is in metaphase II of the second metaphase division when ovulated. Ovum maturation and meiosis are not completed until after fertilization.
2. **Sperm maturation:** After spermatozoa are produced in seminiferous tubules, several maturation processes are necessary before they can participate in fertilization. The first of these occurs in the epididymis, as i) gaining the ability to be motile, ii) gaining the ability to be fertile and losing the cytoplasmic droplet. Spermatozoa cannot participate in fertilization until they have undergone a second maturation process in the female reproductive tract. The maturation process is known as capacitation. A 10-15 days passage through the epididymis is needed for spermatozoa after which fertilization is possible.
    A. **Capacitation:** Chang (1951) and Austin (1951) sperm must reside in the in the female reproductive tract to become capable of fertilization. Capacitation starts in

the uterus. Major site oviduct (isthmic region). Sperm surface components modified and remove by female genital tract secretions.

- Depletion of sperm cholesterol
- Alternation in glycoaminoglycans (GAGs)
- Change in ions as sperm traverse the genital tract

True acrosome reaction-

- Fusion of sperm plasma membrane with outer acrosomal membrane
- Vesiculation over the anterior segments of spermatozoa

False acrosomal reaction- degeneration of sperm

- Release of hydrolytic enzyme- hyaluronidase and acrosin implicated the penetration of the ovum.

3. Interaction of sperm and ovum

Gamete longevity (hr)	Cattle	Horse	Sheep	Swine
Sperm	30-48hr	72-120hr	30-48hr	34-72hr
Ovum	20-24hr	6-8hr	16-24hr	8-10hr

Male ejaculates billion of sperm in to female tract, if sperm are in oviduct just prior to ovulation

4. Sperm-oocyte encounter

A) Fertilization requires three critical events:

a. Sperm migration between cumulus cells (if present)
b. Sperm attachment and migration through the zona pellucida
c. Fusion of sperm and ovum plasma membranes

5. Sperm attachment

Attachment of the sperm head to the zona pellucida is mediated by a receptor sites on the zona surface. Treatment of ova with antizona antibodies or proteolytic enzyme-trypsin blocks sperm attachment. Pre treatment of sperm with antisperm antibodies or glycoprotein can inhibit binding of sperm to zona pellucid, ZP3. Sperm is the sperm receptor (glycoprotein) synthesize by maturing oocyte to which an intact acrosome can bind.

6. Sperm penetration

Penetration of the zona by sperm occurs within 5-15 minutes after sperm attachment.

Enzymes released to penetrate zona

a. At least 9 enzymes released
b. Acrosin – major zona lysin
    - Others act synergistically with acrosin
c. Sperm motility also required for penetration
d. ZP2 may also help in attachment/penetration

7. **Gamete fusion**

   Vitelline membrane may have less specificity than the zona pellucida in binding foreign spermatozoa. The acrosome reaction is a prerequisting for fusion between ova and spermatozoa plasma membrane and zona free ova cannot undergo fusion with non acrosomal activated sperm even though attachment occurs.

8. **Block to polyspermy**

   Immediately foolowing fertilization, the ovum surface change to prevent fusion of additional spermatozoa. Failing their mechanism polyspermic fertilization can result with formation of polyploidy embryos that under embryonic death or its abnormal development.

   a. Polyploid embryos- death or abnormal development
   b. Happens in 1-2% of mammalian fertilizations
   c. Increased by delayed insemination-pigs and sheep

9. Development of pronuclei and syngamy

   Following fusion with egg plasma membrane, the sperm nuclear envelop disintegrats and release chromatin materials undergoes decondensation. The sperm nuclear envelope is rapidly replaced by a new envelop within the ovum cytoplasm forming the pronucleus. The process requires specific components in ovum cytoplasm. Male pronucleus growth factor. Male and female pronuclei migrate to the ovum centre and chromosomal from bith source get intermixed. This is called zygote that strats a new life.

   Syngamy occurs: nuclear envelopes disperse and choromosomes mix

Chapter 5
Herd fertility

Infertility

Primary infertility: When a women becomes pregnant but is unable to carry the pregnancy long enough to deliver a baby.

Secondary infertility: When a couple, after a first pregnancy and labor, is unable to carry the pregnancy long enough to deliver a baby.

Sterility:

Primary sterility: When a couple has not been able to conceive after having had unprotected intercourse for a year.

Secondary sterility: When, after having had a first child, a couple has not managed to achieve a second pregnancy after having had unprotected intercourse for two to three years.

Reproductive failure

Sterility is a permanent factor preventing offspring production and infertility or temporary sterility is the inability to produce viable young within a stipulated time characteristics for each species. The deviation of these natural processes is collectively called reproductive failure. Following are the reproductive failure in female and male animals.

Reproductive failure in female

A. **Ovarian dysfunction:** The two main functions of the ovary, the production of ova and secretion of ovarian hormones, are intimately related and directed toward successful reproduction. Following are the ovarian dysfunctions:
    i. **Anestrus:** seasonal anestrus, anestrus during lactation, anestrus due to aging, anestrus due to nutritional deficiencies, anestrus due to stress, anestrus due to abnormalities of the ovary or uterus.
    ii. **Atypical estrus-**short estrus, prolonged estrus,split estrus, nymphomania and silent estrus.
    iii. **Ovulatory failure-**anovulatory estrus, cystic ovaries.
B. **Disorder of fertilization:** This includes
    i. **Failure of fertilization:** Fertilization failure may result from death of the egg before sperm entry, structural and functional abnormality in the egg or sperm, physical barriers in the female genital tract preventing gamete transport to the site of fertilization.
    ii. **Atypical fertilization:** The complex process of fertilization is subject to several aberrations namely, polyspermy, monospermic fertilization of an egg containing

two female pronuclei, failure of pronucleus formation and gynogenesis or androgenesis. Atypical fertilization may occur spontaneously as a result of aging of the aging of the gametes or elevation of environmental temperature. It has also been induced experimentally by x-rays or the administration of certain toxic substances.

C. **Prenatal mortality:** Prenatal mortality, responsible for most gestation failure, can be divided into embryonic and fetal mortality.

Embryonic mortality: Embryonic mortality denotes the death of fertilized ova and embryos up to the end of implantation. Approximately 25-40% of embryos are normally lost in farm species. Mortality is more common during the early than the late embryonic period.

Fetal mortality: It includes abortion.

D. **Perinatal and neonatal mortality:** Perinatal mortality refers to death of the offspring shortly before, during or within the first 48 to 72 hours of life at normal term. Perinatal mortality includes stillbirths and neonatal mortality.
E. **Disorder of gestation, parturition and puerperium**
   Dystocia: Fetal dystocia, maternal dystocia, fetopelvic dystocia (the pelvis is unusually small or the fetus unusually large. Causes dystocia, in the absence of any obstruction from the cervix or vagina.)
   Retained placenta: Retained placenta or a failure of the fetal membranes to be expelled during the third stage of parturition is a common postpartum complication in ruminants particularly in cattle. Retention of the placenta beyond 12 hours in cattle is considered.
   Hydramnios and hydrallantois: Hydramnios, the excessive accumulation of amniotic fluid is less common than hydrallantois, the accumulation of allantoic fluid. Hydramnios, observed more often in cattle than sheep or swine is associated with certain cranial abnormalities of the fetus.
   Multiple pregnancies: In cattle, horse, sheep and goats the frequency of multiple pregnancies is higher than that of multiple births, owning to the high incidence of abortion and fetal resorption.
   Prolonged gestation: Abnormally long gestations that are due fetal abnormalities in cattle, sheep and swine results from genetic and non-genetic factors.

Reproductive failure in male

The fertility of a male is related to several phenomena:

1. Sperm production
2. Viability and fertilizing capacity of the ejaculated sperm
3. Sexual desire
4. The ability to mate

Followings are the reproductive failure in male animals:

A. Congenital malformations:

i. Segmental aplasia of the wolffian ducts: In this defect, small or large segments of one or both wolffian ducts (epididymis, vas deferences or ampulla) are missing.

ii. Cryptorchidism: The descent of the testes involves the abdominal migration to the internal inguinal ring, passage through the inguinal canal and finally migration within the scrotum. In cryptorchidism, one testis or both testes fall to descend from the abdominal cavity into the scotum.

B. Ejaculatory disturbances:

i. Lack of libido: Libido or sexual desire is an important aspect of male reproductive function. Lack of libido may be heredity or may be originate from psychologic disturbances, endocrine imbalance or environmental factors. Even though seminal characteristics may be satisfactory, fertility may be adversely affected as a result of poor libido.

ii. Inability to copulate: physical disabilities may impede or prevent mating by causing failure in copulatory behavior i.e. mounting, intromission or ejaculation.

C. **Fertilization failure:** Fertilization failure is an important cause of infertility in male that have normal libido and are capable of mating and ejaculation. These are as follows:

- Diseases of testes and accessory glands
- Heat stress
- Breeding techniques

D. **Nutritional and male infertility:** the effects of nutritional restrictions on fertility are more notable in the female than in the male. Nutritional deficiencies delay the onset of puberty and depress production and characteristics of semen in the male. These include: underfeeding, vitamin deficiencies, mineral deficiencies and toxic agent.

E. **Infertility and chromosomal aberrations**: Capacity or reduced capacity is related to defective semen characteristics or to errors breeding techniques.

Economics of poor fertility in cow

- Higher replacement rate (cost of increase replacement rate).
- Change in month of calving with loss of milk revenue (month of calving change).
- Fewer calves per year (long calving interval, loss of calf sales).
- Increased maintenance costs-as calving interval lengthen so the maintenance requirement of the herd increased requiring additional expenditure on fertilizer, purchases roughage or compound feed.
- Higher insemination fees- poor pregnancy rates result in higher number of services per conception and therefore increased fees.

Measures of Reproductive Efficiency

Traits	Definition
First calving	Age (Months)
Days open	Days calving to conception
First service conception rate%	(No. pregnant first service / no. breed first service)×100
Calving interval (days)	Days between successive calving/ Total cows

Services per conception	No. of services in all cows/ Total conception
Pregnancy rate%	(No. of cows pregnant/ Total cows in herd)×100
Calving rate%	(No. of calves born/ Total cows in herd)×100
Net calf crop%	(total calves weaned/ Total cows in herd) ×100

Evaluation of fertility

The parameters and targets commonly used to analyze and evaluate fertility in the dairy herd.

Reproduction parameters and targets for dairy herds

Parameters	Target
Calving-conception interval (av. number of days open)	< 90 days
Calving-1st insemination interval	< 70 days
Conception rate at 1st insemination	> 60%
Number of inseminations per conception	< 1.5
Abortions (Between 450265 days of pregnancy)	< 3%
Culling due to infertility	< 5%
Age at first calving	24 months

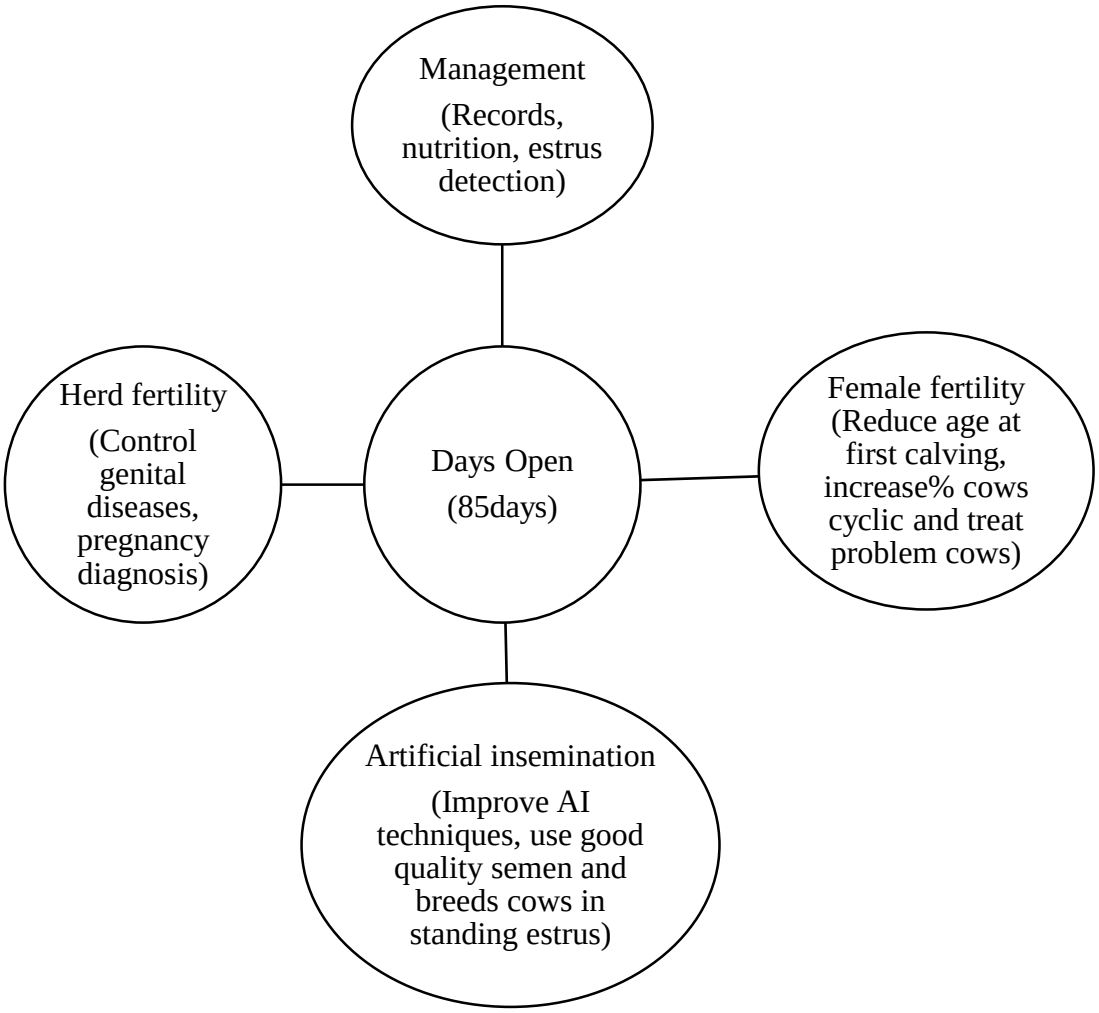


Figure: Methods of improving reproductive efficiency in dairy cattle. For dairy herd herd to achieve a 12 months calving interval, at least 90% of cows should be cycling by 60 days postpartum and conceive within 85 days (days open).

Net calf crop

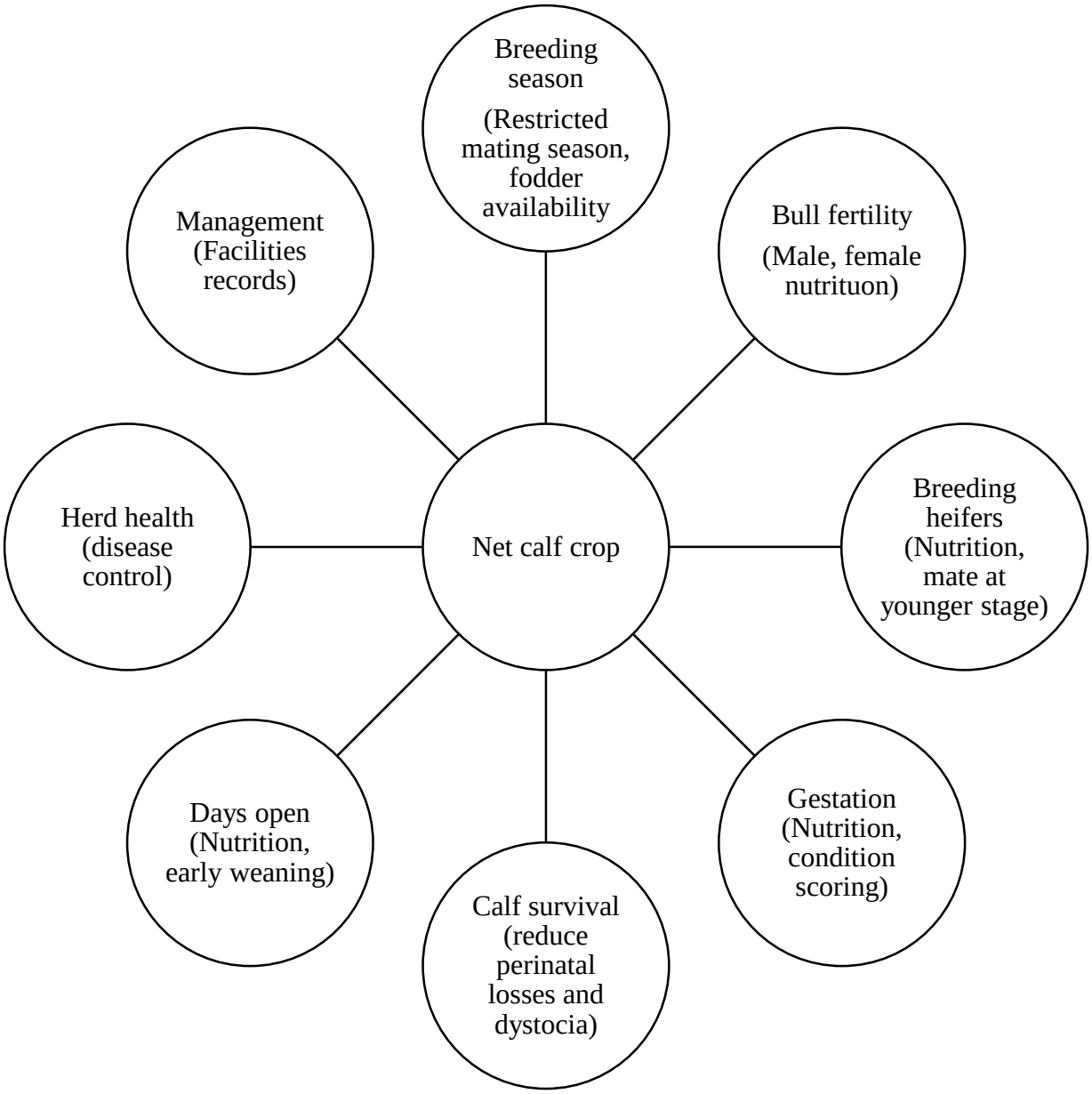


Figure: Methods of increasing the net calf crop in beef cattle

Chapter 6

Artificial Insemination

Artificial insemination, or **AI**, is the process by which sperm is placed into the reproductive tract of a female for the purpose of impregnating the female by using means other than sexual intercourse.

Artificial insemination (AI) is a process by which sperm are collected from the male, processed, stored and artificially introduced into the female reproductive tract for the purpose of conception. AI has become one of the most important techniques ever devised for the genetic improvement of farm animals. It has been most widely used for breeding dairy cattle and has made bulls of high genetic merit available to all.

Several elements of A.I

- Collection of semen from bulls
- Semen is processed and evaluation
- Semen is diluted
- Semen is preserved
- Semen is transferred in to the genital tract of female by means of tube.

History of AI

The concept of A.I is old but there is evidence that the method was known and used by horse breeders in 1300A.D. The first authentic account of the use of A.I was by the Itallian Spallanzani who in 1780 successfully inseminated a bitch. In 1899, Ivanoff of Russia pioneered AI research in birds, horses, cattle and sheep. He was apparently the first to successfully inseminate cattle artificially. Mass breeding of cows via AI was first accomplished in Russia, where 19,800 cows were bred in 1931. Denmark was first to establish an AI cooperative association in 1936. E.J. Perry of New Jersey visited the AI facilities in Denmark and established the first United States AI cooperative in 1938 at the New Jersey State College of Agriculture.

The AI industry has grown tremendously in the United States since its beginning. In 1970, USDA reported that 7,344,420 dairy females were bred artificially, 46% of the female dairy cattle population. Before independence AI introduced in our country on 50th century. After independence, through AI extension project again started on 1975-76.

Advantages and Disadvantages of AI

Some benefits associated with the use of AI

- Enables the widespread use of outstanding sires.
- Leads to improved performance and potential of the national herd and permits coordination of breeding policy on a national basis.
- Permits cross-breeding to change the production emphasis, such as from milk to beef.
- Accelerates introduction of new genetic materials by export of semen and reduces international transport costs.
- Enables the use of deep-frozen semen after the donor is dead.
- Permits use of semen from incapacitated males.
- Reduces the risk of spreading sexually transmitted disease.
- Provides an essential research tool for investigating many aspects of male and female reproductive physiology.
- Animal can be produced economically by artificial means.
- Control breeding is possible by AI

LIMITATIONS

- Poor technique in the manner of AI may result in the spread of reproductive diseases.
- Application of AI should be limited in areas with poor means of transportation and communication.
- Limited value where cattle cannot be kept under close observation.
- Careless use of AI will result in lower breeding value
- It cannot exceed the percentage of conception rate of natural mating.
- AI requires more labor, facilities and managerial skill than natural service.

- Proper implementation of AI requires special training, skill and practice.

Collection, Extension and Storage of Semen

One obvious factor which determines degree of success of AI is the quality of the semen used. Much has been learned about factors affecting semen quality and methods of evaluation and means of maintaining quality through lengthy storage. The commercial AI industry has a tremendous responsibility to sell only high quality semen. Unquestionably, they are fulfilling this obligation.

Collection of Semen

There are at least 4 recognized method of semen collection. These ares-

1. Artificial vagina (A.V) method
2. Electro-ejaculation method
3. Massage method
4. Collection of semen from vagina of female when she is bred.

Several methods of obtaining semen have been developed. The artificial vagina method is most widely used today for the collection of bull semen. The bull is allowed to mount a teaser cow and ejaculates when the penis is directed into the artificial vagina. The artificial vagina consists of a firm cylindrical tube with a thin-walled rubber lining. The jacket formed is filled with warm water. A rubber funnel connected to a collection receptacle is attached to one end of the cylinder. When the jacket is properly filled and the artificial vagina lubricated and properly applied, this method of semen collection is highly successful.

Cleanliness must be practiced to avoid contamination and deterioration of semen quality. Proper and careful treatment of the bull is essential to bring about adequate precollection stimulation which will increase quantity and quality of semen obtained.

Obviously the collection of semen from a bull is a specialized skill and should be attempted only by those with the proper equipment, training and experience. Adequate facilities for controlling the bull and teaser animal must be maintained so that danger of injury to personnel as well as the animals is minimized.

Semen evaluation

Volume: Young bull usually start 2-3ml

Mature bull start 8-14 ml

Breed, age, level of nutrition, climate, teasing time, technical skill of the operator, management, frequency of collection, temperaments of the male contribute to the various observed in the volume of semen collected.
Normal bull semen is milky white in color.

Motility

0-No progressively motile sperm
1-Sluggish motility, slow forward progress
2-Motility somewhat sluggish, still progressive but not rapid and flashy
3-Intermediate motility, not the best but fairly rapid progressive motility
4-Maximum progressive motility, very rapid and flashy.

Semen Extension/ Semen Diluents

The main reason for extending (diluting) semen is to increase the number of females serviced from one ejaculation. A normal ejaculate from a dairy bull will contain 5 to 10 billion sperm which can be used to inseminate 300 to 1000 cows if fully extended.

There are several good semen extenders. Those made from egg yolk or pasteurized, homogenized milk are two of the most widely used. A good extender not only adds volume to the ejaculate but favors sperm survival and longevity. Dilution rate depends on quality of the ejaculate--number of sperm cells, percent alive and mobility. As few as 12 million sperm per insemination have given good conception rates.

Penicillin and streptomycin are added to semen extenders. These antibiotics inhibit bacterial growth and reduce danger of spreading diseases such as vibriosis.

Function of semen diluents

Semen diluents provide nutrients for the metabolic process of sperm, cold-shock protection, buffer effects against the lactic acid produced by sperm metabolism, a reducing substance for the

protection of certain enzymes and (certain diluents only) co_2 gas which stops motility and reduces sperm metabolism. They also help to maintain proper osmotic pressure and proper mineral balance.

Composition of diluents:

Ingredients	Bull	Ram	Boar	Stallion
Buffer (% by volume)	80	80	70	70
Egg yolk (% by volume)	20	20	30	30
Penicillin (units/ml)	1000	-	-	100
Dihydrostreptomycin (µg/ml)	1000	1000+	1000	200

Milk is a satisfactory diluent for bull, Ram boar and stallion semen

Determine the dilution rate of any particular sample of bull semen follows:

Volume of ejaculate= 8 ml

Concentration of sperm= 1200000000 per ml (1200 million/ml)

Percentage of motile sperm= 70

Then. 1 ml of semen contains= 1200000000 ×70/100

= 840000000 live sperm

Number of motile sperm required in 1 ml. of diluted bull semen= 8000000

Thus dilution rate= 840000000/8000000= 105

Then 8 ml of semen can be diluted (8×105) = 840 ml

Ref: Reproduction in farm Animals by E.S.E. Hafez

Semen Storage

The discovery that bull semen could be successfully frozen and stored for indefinite periods has revolutionized AI in cattle. In 1949, British scientists discovered that addition of glycerol to the semen extender improved resistance of sperm to freezing. Glycerol acts to remove water from the sperm cell prior to freezing and prevents the formation of cellular ice crystals which would damage the sperm. There are two methods of freezing and storing semen: dry ice and alcohol (-100 degrees F) and liquid nitrogen (-320 degrees F/ -195ºC). Liquid nitrogen is preferred because there is no evidence of fertility deterioration with age. Fertility gradually declines in semen stored in dry ice-alcohol.

Frozen semen can be stored indefinitely if proper temperature is maintained. A recent report told of a calf born from frozen semen stored for 16 years. Fresh, liquid semen can be successfully stored for 1 to 4 days at 40 degrees F/5 ºC.

Semen is usually stored in glass ampules. Other methods appear promising, particularly the French-straw. Several AI organizations have gone to this method exclusively.

Artificial coloring is frequently added to semen extenders in order to distinguish one breed from another. Complete identification of the bull is required on each individual semen container.

2. Artificial Insemination Techniques

To successfully practice artificial insemination , it is necessary to have information on the following: a) Sign of estrus b) Time of onset of estrus and ovulation c) duration of estrus d) Optimum time to inseminate e) Expected rate of conception, if it is not possible to inseminate at the optimum time f) proper insemination technique

The technique of inseminating a cow is a skill requiring adequate knowledge, experience and patience. Improper AI techniques can negate all other efforts to obtain conception. Semen must be deposited within the tract of the cow at the best location and at the best time to obtain acceptable conception rates.

Early methods of AI involved deposition of the semen in the vagina, as would occur in natural mating. Those methods are not satisfactory. Fertility is low and greater numbers of sperm are required. Another method which gained popularity was the "speculum" method. This method is easily learned, but proper cleaning and sterilizing of the equipment is necessary, making it more impractical to inseminate than with the rectovaginal technique which is the most widely used AI method today.

In the rectovaginal technique a sterile, disposable catheter containing the thawed semen is inserted into the vagina and then guided into the cervix by means of a gloved hand in the rectum. The inseminating catheter is passed through the spiral folds of the cow's cervix into the uterus. Part of the semen is deposited just inside the uterus and the remainder in the cervix as the catheter is

withdrawn. Expulsion of the semen should be accomplished slowly and deliberately to avoid excessive sperm losses in the catheter. The body of the uterus is short; therefore, care should be taken not to penetrate too deeply which might cause physical injury. In animals previously inseminated, the catheter should not be forced through the cervix since pregnancy is a possibility. Since research data show little variation in conception rates when semen is placed in the cervix, uterine body or uterine horns, some people recommend incomplete penetration of the cervical canal and deposition of semen in the cervix.

The rectovaginal technique is more difficult to learn and practice is essential for acceptable proficiency but the advantages make this method of insemination more desirable than other known methods. With practice, the skillful technician soon learns to thread the cervix over the catheter with ease. If disposable catheters are used and proper sanitation measures are followed, there is little chance of infection being carried from one cow to another.

3. Timing of Insemination for Maximum Conception

Maximal conception is obtained when cows are inseminated between midestrus and the end of standing estrus, with good results up to 6 hours after estrus.

Success in insemination timing is dependent upon a good heat detection program. In large herds, this means assigning individual responsibility for heat detection and a continued education program for labor. A successful heat detection program and subsequent proper timing of insemination will pay dividends in increasing reproductive efficiency.

Proper timing of insemination.

Cows showing estrus	**Should be Inseminated**	**Too late for good results**
In morning	Same day	Next day
In afternoon	Morning of next day or early afternoon	After 3 p.m. next day

Insemination requirements and related phenomena in farm mammals

Item	Cattle	Sheep	Swine	Horses
Frequency of semen collection (per week)	3-5	7-25	3-5	7-10
Characteristics of average ejaculate				
Volume(ml)	8	1	215	125
Sperm concentration (million/ml)	1200	3000	270	120
Total sperm/ejac. (million)	9600	3000	58000	15000
Motile sperm (%)	70	75	60	70
Morphological normal sperm (%)	80	90	60	70
Recommended diluents	yolk-citrate	yolk glucose-citrate	yolk-glucose-bicarbonate	glucose-gelatin
Storage temperature for liquid semen(^{0}C)	5	5	15	15
Rate of dilution(1 ml semen diluted to---ml)	105	9	4	2
Storage of liquid semen (days)	4	1/2	1	1
Optimum time to inseminate	middle or end of estrus	toward end of estrus	first/second day of estrus	third day of estrus
Dose of insemination (volume,ml.)	1	0.2	50	20-40
motile sperm no., million	8	50-60	2000	1500

Deposition of semen	cervical	cervical	cervical	Uterine
No. of possible females breed/week	800	40	17	7
Conception on Fist insemination (% pregnant)	65	70	70	65

*One or two days of rest should be provided each week

Some commonly observed measurements of the age at puberty, together with characteristics of the estrous cycle in the large farm species

Phenomena	**Cow**	**sheep**	**pig**	**Horse**
Age at puberty (months)	4-14	7-10	4-7	15-24
Length of estrous cycle (d)	Avg.21	Avg.17	Avg.21	Avg.21
Length of luteal phase(d)	17-18	14-15	15-16	14-15
Duration of oestrus	Avg.17 hrs	Avg.35 hrs	Avg.2 days	Avg.6 days
Timing of ovulation	2-26 hrs after end of oestrus	12-24 hrs before end of oestrus	16-48 hrs. from beginning of oestrus	24-48 hrs. before end of estrus
Optimum time to inseminate	middle or end of estrus	toward end of estrus	first/second day of estrus	third day of estrus

Life span of sperm & ovum in the genital tract:

Class of animal	Life spam of sperm	life span of ovum
Bull/cow	28-30 h	20 hr
Ram/ewe	24-40 h	24 h
Stalion/mare	4-6 days	4 h
Boar/sow	24-40 h	24 h
Man/Woman	22-72 h	24 -48 h

Advantages of Artificial Insemination over Natural Service

Artificial insemination (AI) is one of the most efficient tools accessible to dairy farmers to improve productivity and profitability of dairy enterprise. In artificial insemination the bulls of superior quality can be efficiently exploited with the least concern for their location in faraway places. There are a lot of advantages of AI over natural services with bulls. They are as follows:

- *Boosts efficiency of bull usage*: During natural mating, a bull will donate much more semen than is theoretically needed to make a pregnancy. On the other hand, collected semen can be diluted and extended to make hundreds of semen doses from a single ejaculate which can be easily carried one place to another, promoting multiple inseminations in females in different geographical locations and semen can be stored for long periods of time
- *Cost Effectiveness:* No necessity of maintenance of breeding bulls. Hence, the expenditure on maintenance of breeding bull is saved.
- *Checks disease transmission:* Natural mating allows the transmission of venereal diseases between males and females. On the other hand, for AI, semen is regularly tested for its quality, possible infections hence allows checking of the spread of certain venereal diseases. Eg: contagious abortion, vibriosis.
- *Promotes Breeding Efficiency:* By routine examination of semen after collection and frequent checking on fertility make early detection of inferior bulls and better breeding efficiency is warranted.
- The progeny testing can be employed at an early age.
- The semen of an elite bull can be used even after the death of that sire.
- It makes possible the mating of animals with great variations in body size with no injury to either of the animal.
- It is useful to inseminate the cows denying to stand or accept the bulls at the time of oestrum.
- Useful in maintaining the perfect breeding and calving records.
- Artificial Insemination enhances the rate of conception.
- Artificial Insemination allows the use of old, heavy and injured sires.
- Artificial Insemination when linked to oestrous synchronization programme, can promote a more consistent, uniform calf crop production.

Semen Physiology & Sperm Biology

Semen: Semen is the combination products of the testes and the ex-current ducts, and the secretions of the accessory glands. It contains spermatozoa and varying proportions of fluids and of secretions from the glands that line the male reproductive tract and the glands that empty into it.

Contributions of the various parts of the reproductive tract to semen: The testes contribute spermatozoa to semen. They also contribute various cellular fragments that have been cast off by the spermatozoa in their maturation and fluids that have not been resorbed in the outward passage of the spermatozoa. The epididymis contribute three substances to semen-glyceryl-phosphoryl-choline, carnitine, and sialic acid. A high level of lactate is also present in epididymal secretion and apparently serves as an oxidizable substrate for the spermatozoa when they are transported through that organ. The secretions of the ampulla also contribute to the fluid volume of semen. The secretions of the ampulla contain citric acid and fructose. Most secretions that contribute to the fluid volume of semen come from the prostate gland, the seminal vesicles, and the Cowper's glands, of which the seminal vesicles contribute the largest position. The prostate gland is the source of sperm-cell antiagglutin. The seminal vesicles are the primary source of seminal fructose, citric acid, and ascorbic acid.

Physical and Chemical properties of semen: Freshly ejaculated, normal bull semen containing active, motile spermatozoa exhibits a characteristic wave motion which, against the confines of a glass receptacle, gives the appearance of "boiling." The numbers of sperm cells in a given volume of seminal fluid affect its appearance. A sample that is clear and translucent is low in sperm-cell number, whereas one that is opaque and thick is usually relatively high in sperm-cell concentrations. Followings are the physical and chemical properties of semen.

1. **Color:** Most bull semen is milky-white, varying to a cream color. Up to 10% of bulls produce semen that is normally yellow.
2. **Volume:** The volume of ejaculate varies from bull to bull and within each bull. In general, the volume increases with the age and the body size of the bull and changes with his general reproductive health and vigor and the frequency of his use. Young bulls just coming into service produce as little as 1 to 2 milliliters or less of semen per ejaculate, whereas fully mature, vigorous bulls weighing up to 900 kilograms or more may produce 10 to 15 milliliters or more of semen per ejaculate. Continuous observations on the same bulls have shown a trend toward smaller volumes after full maturity.
3. **Sperm-cell concentration:** The number of spermatozoa per unit volume of bull semen varies from zero in complete azoospermia to over 3 billion (3000 x 10 ^6) cells per milliliter in occasional very dense samples. The upper limits of sperm-cell concentration usually range from 2000 x 10^6 to 2200 x 10^6 cells per milliliter. A number of factors contribute

to the variability in sperm-cell concentration. In general, the concentration varies with the sexual development and maturity of the bull, with the feeding regime, and with the reproductive health and size of the testes. In addition, there are real differences in sperm-cell concentration among bulls, among different age groups of bulls, at different seasons of the year, and in different geographical locations.

4. **Density and Viscosity:** The absolute density the specific gravity of bull semen ranged to be 1.036+-0.0086. There have positive correlation with sperm-cell concentration. The viscosity of semen increases with increasing sperm-cell concentration.
5. **Osmotic Pressure:** The osmotic pressure exerted by semen on the sperm cells it contains, and on the cells lining the portion of the reproductive tract in which it is found, has important physiologic consequences. The osmotic pressure exerted by a fluid depends on its concentration of particles, including ions, small nonelectrolyte molecules, and the larger colloids.
6. **pH:** The pH of freshly ejaculated bull semen depends on the varying proportions of the several secretions involved. Most normal samples are on the acid side of neutral, varying from about pH 6.5 to 6.9 with a mean of about 6.75, hut the pH varies over a wide range from about 6.0 or lower to 8.0 or slightly higher. Good quality semen is usually more acid (lower pH) than semen with lower sperm-cell concentrations. Poor-quality semen contains a proportionately larger amount of fluid from the urethral and accessory glands.
7. **Buffering Capacity:** Buffering capacity is the chemical ability of a fluid to absorb acid or alkalis with little change in pH. Bull semen is relatively highly buffered at pH levels below 5.5 and above 9.0. It is only moderately well buffered at pH 5.5 to 6.5 and at pH 8.0 to 9.0. It lacks buffering capacity between pH 6.5 and pH 8.0.
8. **Electrical Conductivity:** Owing to the presence of acid, base, or electrolyte ions in bull semen, it is conductor of electric current.

The Inorganic Constituents of Semen:

1. **Bulk Cations**: Sodium, Potassium, and Calcium make up most of the inorganic mineral elements in semen, and so they have been referred to as the bulk cations in semen.
2. **Trace Minerals:** A number of other mineral elements have been reported in semen in relatively low concentrations. They include boron, magnesium, iron, copper, and zinc.
3. **Carbon Dioxide Content:** A carbon dioxide level in bull semen of 16 ml/100 ml.

The Organic Constituents of Semen:

1. **Dry Matter:** The dry-matter content of whole bull semen was found to be 9.98+- 1.77 percent.
2. **Carbohydrates:** The carbohydrate contents of semen reported to be glucose, and fructose.
3. **Other Reducing Substances:** Ascorbic acid was found in bull semen at levels ranging from 3mg/100ml to 8mg/100ml, for normal bulls, and at even lower levels for bulls with

low fertility. Bull semen contains no ergothioneine, which is the primary source of reducing sulfhydryl groups in boar semen.

4. **Polysaccharide:** Ram semen contains a small amount of carbohydrate material which resembles glycogen in that it precipitates in ethanol but is alkali-resistant.
5. **Organic Acids:** The organic acid content in semen depends on the concentration and activity of the sperm cells in the semen and the length of time the spermatozoa are in the presence of the seminal materials before analysis.
6. **Nitrogen-Containing Compound:** Amino acids, proteins and ammonia are the general nitrogen containing compounds in semen.
7. **Phosphorus-Containing Compounds:** Bull semen contains relatively high levels of phosphorus, most of which is in an organic form. Bull semen also contains phosphagens, phosphorus compounds linked to creatin, to arginine, or to both.
8. **Vitamins:** Bull semen contains, in addition to ascorbic acid, a number of the B-complex water-soluble vitamins.
9. **Enzymes:** Bull seminal plasma contains a number of enzymes, some of which may be diffused from the sperm cells.
10. **Other Components:** Bull semen contains about 1 microgram of adrenaline or noradrenaline per milliliter. One important group of compounds present in seminal plasma is the prostaglandins.

Management of an Artificial Breeding Programme

1. Limitation

- Poor technique in the manner of A.I. may result in the spread of reproductive diseases.
- Application of A.I. should be limited in areas with poor means of transportation and communication.
- Limited value where cattle can not be kept under close observation
- Careless use of A.I. will result in lower breeding value.
- It con not exceed the percentage of conception rate of natural mating % conception rate

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2. Factors Affecting Efficiency in A.I.
    - ❖ Competence of A.I. Technician
    - ❖ Fertility of the semen
    - ❖ Fertility of the cow
    - ❖ Herd management
    - ❖ Planning and Conduct
    - ❖ Record Keeping

a. Technician & His Job

What every A.I. technician must know:

A. Equipment's & Supplies in A.I.
B. Heat detection
C. Best time to Breed
D. Semen Storage & handling
E. Preparation of Semen for Insemination
    1. Transfer of straw from flask
    2. Thawing Procedure
    3. Loading the A.I. gun
F. Insemination of Cow
    1. Method of A.I.
    2. Procedure of A.I.
G. After Insemination
    1. Washing & cleaning
    2. Recording
    3. Release the animal
H. Inseminator's Hygiene

B. Fertility of The Semen

1. The genetic:

- Determination of fertility in the bull.
- Variation between and within the breeds.
- Always use only those bulls, which are known to have good fertility.

Semen handling:

- Maximum precurtions are to be take to be to ensure that semen order are prepared in such a way as to minimize the chance of damage to the sperm.

Genetic makeup of the cow:

- Particular bulls are not "cliked" with certain lines of cows.
- (Animals carrying recessive lethal factors, which result in early embryonic deaths.)

C. Fertility of the cow

i) Genetic: absence or lack of development of organs.

ii) Hormonal: Imbalances caused by cystic ovaries, persistant corpuslutium etc.

iii) Nutritional disorder.

iv) Infectious diseases (Brucellosis, Vibriosis, Leptospirosis).

v) Abnormalities of the tract.

vi) Other nonspecific diseases of reproductive organs.

vii) The heat cycle:

- Lactation: Suckling reduces the supply or release of gonadotrophins (insufficient LH).
- Pregnancy: Regular program for pregnancy checking.
- Silent heat
- Dystokia, retained placenta, twin calvin's or infections
- Puberty age of heifer: Rate of growth.
- Old age
- Male functions of the reproductive tract.

D. Herd Management

- Select animals best suited for upgrading the herd.
- The cattle raiser should have all his cows bred artificially.
- Have the selected animals diagnosed for pregnancy.
- Vaccination and dewarming must be done before breeding starts.
- Remove all bulls from the group at least 2 months before A.I. starts.
- Feed the cow well and keep them as quite as possible.
- Identify the animals with eartag and brand marking.
- Teach the farmers how to identify cows in heat if necessary.
- Water should be available at all time.

E. Planning and conduct

1. The aim of the program:

- Economically viable
- Personal satisfaction of the farm

Breeding plan:

- Progeny testing program
- Wider use of tested bull
- Grading up to new breed
- Crossbreeding
- Production of breeding bull from nucleus herd

Factors affecting implementation:

- Availability of finance

- Breeding society rules
- Technical knowledge of A.I.
- Development of facilities
- Availability of breeders
- Availability of semen

Record keeping

- Date of birth of the cow
- Sire & dam of the cow
- Milk record of the cow
- Milk record of the cows dams, sires dam if available
- Date of Estrous
- Date of A.I. (1^{st} repeat etc.)
- Pregnancy record
- Calving record
- Breeding trouble and infertility problem if any
- Disease history of the cow

Components of semen extenders

The basic components of extenders for freezing of bovine spermatozoa are

- Buffer
- Fructose
- Glycerol
- Egg yolk
- Antibiotics

Buffer:

- A variety of organic and inorganic buffers have been used for deep freezing of semen.
- But an ideal buffer should prolong the life if spermatozoa at room temperature penetrate cells and acts intracellular buffer, less toxic in the critical temperature during freezing and better clarity under microscope.
- The most commonly used diluent in most of the laboratories for deep freezing of semen is the inorganic buffer called tris buffer.

Tris is commonly called as universal diluents which prolongs the life of sperm at room temperature, penetrate sperm and acts as intra cellular buffer, less toxic at the critical low freezing temperature.

Preparation of buffer:

- Weigh the required chemicals accurately in an analytical balance.
- Mix the chemicals in double distilled water thoroughly in a conical flask.
- Adjust the p^H of buffer to 6.5-7.0
- Boil the contents for few minutes in a water bath.
- Cool and add antibiotics.
- Store the buffer in a refrigerator.

Fructose:

Addition of fructose provides glycolysable substrate for sperm, prevent agglutination, and maintain required osmotic tension and electronic balance and gives better cryoprotection during deep freezing.

- Fructose added in the diluent is the major energy source for the sperm

Glycerol:

Glycerol is the most widely and commonly used cryoprotective agent for bull spermatozoa.

- It was the spectacular discovery of polge in 1949 that showed that the death of spermatozoa could be avoided if the cells were suspended in a medium containing glycerol.
- The possible modes of action of glycerol are:
    - Modifies the size and shape of ice crystals formed.
    - Binds water and decrease freezing point of solution and less ice is formed.
    - Acts through salt buffering mechanism.
    - Reduces solute concentration.
    - Prevents denaturation of proteins and rupture of plasma membrane.

Egg Yolk:

- When spermatozoa is cooled to 5^oC they are subjected to cold shock, which causes leakage of intracellular enzymes and other materials present in the spermatozoa.
- This damage can be prevented by providing addition of lecithin, protein, lipoprotein and similar compounds present in the egg yolk.
- In addition the glucose, proteins and vitamins present in the egg are utilized by the spermatozoa and also protect the enzymes
- The value of the yolk of the hen's egg as a diluting medium in semen preservation was found by philips during 1939.
- It helped in rapid growth in recent years of artificial insemination throughout the world.

Egg yolk protects lipoprotein sheath of sperm against cold shock.

- The active principle in yolk responsible for this is now thought to be lecithin or a similar phospholipid occurring either free or in combination with protein.
- It contains glucose, proteins, vitamins and required viscosity index which may be of advantage to the sperm cells.
- The hydrogen peroxide formed, which is toxic to the sperm, may be destroyed by additions of the enzyme catalase to the yolk diluents, under anaerobic conditions the problem does not occur.

Preparation of Egg Yolk

- Egg yolk is commonly added with buffer at the ratio of 1:4 (20%) to prepare the extender for dilution.
- Unfertilized egg is used for this purpose.
- The egg should be purchase from disease free, known flock.
- Fresh egg immediately after laying is purchased
- The egg immediately after receiving is wiped with dry cotton and stored at refrigerator at 4-5^{o}C
- Maximum it can be stored use it is cleaned with swab of 70% alcohol
- It is opened at the narrow end with the help of a sterilized foreceps.
- The white of an egg (albumin) is drained off with the help of an egg yolk separator and yolk is placed on a sterile filter paper.
- If required yolk may be transferred to another filter paper to clear traces of albumin.
- Then the vitelline membrane is punctured with a sterile glass rod and yolk is allowed to fall in a sterilized graduated cylinder directly at the bottom without touching the walls.
- The required quantity is prepared and added with autoclaved buffer (added with glycerol) and mixed with magnetic stirrer.

Overview of Artificial Insemination in Poultry

- Artificial insemination (AI) is widely used to overcome low fertility in commercial turkeys, which results from unsuccessful mating as a consequence of large, heavily muscled birds being unable to physically complete the mating process. This is a serious and costly problem in the production of commercial turkey hatching eggs. In most commercial chicken production systems in the USA, it has not been necessary to implement AI programs because natural mating results in adequate fertility levels, but AI is routinely used in special breeding work and research. However, as managing commercial broiler breeders to maximize fertility becomes more challenging, the use of AI in commercial poultry operations outside the USA is becoming more common. Certainly, the use of AI in chickens, as in turkeys, can improve fertility; however, the cost of implementing AI on a large scale is often cost prohibitive.

- Collecting semen from a chicken or turkey is done by stimulating the copulatory organ (the phallus) to protrude by massaging the abdomen and the back over the testes. This is followed quickly by pushing the tail forward with one hand and, at the same time, using the thumb and forefinger of the same hand to apply pressure in the area and to "milk" semen from the ducts of this organ. Semen flow response is quicker and easier to stimulate in chickens than in turkeys. The semen may be collected with an aspirator (turkeys) or in a small tube or any cup-like container. In turkeys, the volume averages ~0.35–0.5 mL, with a spermatozoon concentration of 6 to >8 billion/mL. In chickens, volume is 1–2 times that of turkeys, but the concentration is about one-half. Collected semen is usually pooled and diluted with an extender before use.
- Chicken and turkey semen begins to lose fertilizing ability when stored >1 hr. Liquid cold (4°C) storage of turkey and chicken semen can be used to transport semen and maintain spermatozoal viability for ~6–12 hr. This short-term storage of semen is common in turkeys, while not as common in chickens. When using liquid cold storage for >1 hr, turkey semen must be diluted with a semen extender at least 1:1 and then agitated slowly (150 rpm) to facilitate oxygenation; chicken semen should be diluted and then cooled—agitation is not necessary. Chicken and turkey semen may be frozen, but reduced fertility limits usage to special breeding projects. Under experimental conditions, fertility levels of 90% have been obtained in hens inseminated at 3-day intervals with 400–500 million frozen-thawed chicken spermatozoa.
- Several commercial semen extenders are available and are routinely used, particularly for turkeys. Extenders enable more precise control over inseminating dose and facilitate filling of tubes. Results may be comparable to those using undiluted semen when product directions are followed. Dilution should result in an insemination dose containing ~300 million viable spermatozoa for turkeys. However, the number of spermatozoa inseminated will range from 150–300 million viable cells depending on the age of the turkey hens inseminated. In chickens, the number of diluted semen inseminated will range from ~100–200 million sperm cells per insemination. Producers usually determine the spermatozoa concentration and dilute the semen to obtain the appropriate sperm cell concentration for either the turkey or chicken.
- For insemination, when holding the hen upright, pressure is applied to the abdomen around the vent, particularly on the left side. This causes the cloaca to evert and the oviduct to protrude, so that a syringe or plastic straw can be inserted ~1 in. (2.5 cm) into the oviduct and the appropriate amount of semen delivered. As the semen is expelled by the inseminator, pressure around the vent is released, which assists the hen in retaining sperm in the vagina or oviduct. When inseminating undiluted turkey semen, the high sperm cell concentration allows for 0.025 mL (~2 billion spermatozoa) to be inseminated at regular intervals of 7–10 days, yielding optimal fertility. In chickens, because of the lower spermatozoon concentration and shorter duration of fertility, 0.05 mL of undiluted pooled semen, at intervals of 7 days, is required. The hen's squatting behavior indicates receptivity and the time for the first insemination. For maximal fertility, inseminations may be started before the initial oviposition in turkeys, whereas this is not necessary in chickens. Fertility tends to decrease later in the season; therefore, it may be justified to inseminate more frequently or use more cells per insemination dose as hens age.
- Artificial insemination has been widely applied to poultry. Semen collection, processing and AI have been reviewed by Sexton (1979) and Lake (1986) and more recently by

Donoghue and Wishart (2000). Pioneers in the poultry field were Burrows and Quinn (1937) who developed the method of abdominal massage and pressure to collect semen. With the ease of the collecting poultry semen and proximity of hens on large breeding farms. AI is used extensively with freshly collected semen. It is used 100% for turkey breeding because mating is difficult. Freshly collected semen was among the first type of semen to be frozen (Shaffiner et al. 1941; Polge et al. 1949). However, cryopreserved poultry sperm are less fertile and freezing poultry sperm still experimental (Gill et al. 1999).

Artificial insemination

Artificial insemination (AI) is the process by which semen from male bird is collected and then introduced to females for the purpose of fertilizing eggs.

The main objectives insemination in poultry are:

a) To place the required dose of semen into the oviduct of the female so that it is deposited near the sperm storage glands and
b) To carry out the AI process with due regard to the best health and welfare of the breeder females thereby achieving the highest fertility levels possible.

Biologically, after deposition of semen in the oviduct the semen will enter the sperm storage gland, situated at the junction of the vagina and the shell gland and from here the spermatozoa will make their way up the oviduct to a second storage site situated at the junction of the magnum and infundibulum. The passage of an ovum into the infundibulum stimulates spermatozoa activity and fertilization of the ovum by one sperm takes place.

Some of the advantages that have been claimed for artificial insemination in the past have been:

- **Increased mating ratio**: In a flock it is usually one cockerel mated to six to ten hens. With artificial insemination it is claimed this ratio could be increased fourfold. In both cases it depends on the strain and breed of the birds. In my commercial farming days with white leghorn cockerels I used from five to seven cockerels with a pen of a hundred hens. When heavy meat birds came along it was about one cockerel to about eight or ten hens in a large flock.
- **Use of older males from outstanding performers**: Older male birds that have been flock improvers can be used for several generations. Whereas under natural mating their useful life is limited.
- **Able to use an injured bird**: Valuable male birds that have been injured in the leg can still be used for artificial insemination.
- **Elimination of preferential mating**: When there is poor fertility cause by preferential mating it can be eliminated.

- **Laying cages can be used**: Laying cases are no longer a problem when fertile eggs are needed. Selected hens can be inseminated and remain in the cage. The exact pedigree of the chickens hatched from these fertile eggs is known. Several commercial farms used colony cages with several hens and one rooster, fertility always seemed to be a problem; artificial insemination did solve this problem for some, until this tyoe of housing went out of favor generally. Although there is still one large farm only an hours drive from my office that still uses this system for its breeding stock. See the photo of a colony cage from my farm, which I used for layers but not for breeding purposes.
- **Successful cross breeding**: Usually cross breeding is very successful under natural conditions, but sometimes there is a kind of color discrimination, some hens will not mate with a male of a different color unless they have been reared together. During my commercial poultry farming when the white leghorn Australorp cross was the layers of the day, I always reared the white leghorn cockerels with the Australorp pullets' right from the day old onwards. This practice produced very good fertility in this cross. But for small breeders or poultry fanciers rearing them together is not always possible, artificial insemination could be a solution to a fertility problem.

Why we do AI?

Increased mating system:

- In a flock usually one cockerel mated to six to ten hens.
- With artificial insemination ti is claimed this ratio could be increased fourfold.
- Depends on the strain and breed of the birds.

Use of older males with outstanding performance: Older male bird that have been flock improvers can be used for several generations. Whereas under natural mating their useful life is limited.

Able to use an injured bird: Valuable male birds that have been injured in the leg can still be used for artificial insemination.

Successful cross breeding: Very successful under natural conditions but sometimes there is a kind of color discrimination. Some hens will not mate with a male of a different color unless they have been reared together. In AI cross breeding is quite easy and successful.

When to use AI: Chicken breeders may be disappointed when their better birds fail to reproduce. The birds may not mate because of shyness, physical limitations and lack of interest or social incompatibility. Unsatisfactory nutrition, age of breeders, management conditions, egg collection and holding practices and incubation procedures can also influence production.

If birds do not reproduce when other conditions are adequate, artificial insemination may be the answer. It is relatively simple and can be used for many kinds of birds, but it requires practice and the proper equipment. It cannot, however, overcome poor management practices, poor health, genetic lethal or differences.

Artificial insemination is more an art than a science. The procedure is not highly technical, but basic knowledge and appreciation of the bird's anatomy is necessary. Success depends largely on the patience and skill of the inseminator. Wild-bird and waterfowl breeders should practice first with some common poultry type – Cornish bantams would be excellent choices.

The male

For best results, the male used for artificial insemination:

i. Must be mature, healthy and physically normal.
ii. Must be sexually active. This is especially important in birds that have a limited season. Light stimulation may be used to control the season in some varieties.
iii. Must be tame or at least not terrified when restrained or handled.
iv. Should be free from external parasites. Some parasites irritate the vent area, making male organ exposure difficult and painful to the bird.
v. Should be kept apart from, but preferably in sight of females.
vi. Should not be subjected to extreme temperatures or allowed to become overheated.

Procedure (Male)

Experts have developed several ways to hold males for semen collection. Techniques may require one or two persons. The following two-operator method works well and reduces fright or feather damage to the bird.

Hold the male with his head toward the operator and with the keel lying in the palm of the left hand. Secure the right leg between the first and second fingers. To make larger birds more comfortable, hold the left leg between the second and third fingers. Stroke the back from midpoint toward the tail the right hand, massaging the abdomen from below with the fingers of the left hand. After several vigorous strokes, transfer the right hand from the back to a position where the thumb and forefinger can apply pressure to either side of the vent. Simultaneously apply pressure to the abdomen with the fingers of the left hand. A slight milking action may increase semen flow. An assistant should catch the semen in an eye cup or other small smooth-edges vessel. In some instances, especially with waterfowl, the copulatory organ may not extend completely. Semen collection is still possible, however, as it flows over the surface of the partially averted vent.

Points to remember:

- ❖ Stimulate males and collect semen immediate after catching. Holding a male, even a tame one for only a few minutes may interfere with collection.
- ❖ Successful semen collection usually results from an experienced operator and an experienced subject.
- ❖ First attempts at working inexperienced males often produce unsatisfactory results. Some males pass feces or urates as they discharge semen. Try to collect only semen,

contaminated semen usually produces poor results. Withhold water and feed four to six hours before collection to lessen chances for contamination.

- ❖ The volume of semen discharged varies from bird to bird. Most males produce between 0.1cc and 0.44 cc during each successful collection.
- ❖ Individual males vary considerably in time needed to replenish their semen supply. Normally, however, you can collect semen every two to four days without harming the birds.
- ❖ Use the semen as soon as possible. It can be held one or two hours without great loss in fertilizing capacity, or longer under controlled conditions. Don't allow the semen to dehydrate and keep it below the body temperature of the male that produced it.

Procedure (Female):

When handling and exposing the female, remember the hen is delicate and must be treated gently. Hold and stimulate her in much the same way as the male. As the operator applies pressure after the preliminary stroking and message, the vent exerts and an orifice appears on the left side. It may be a round rosette or a cleft or skin over fold. An assistant should place the semen ¼ to 1 inch deep into this opening with a 1cc syringe, a medicine dropper or similar device. When making individual mating- one male with one female-use the entire semen collection. Various studies show, however, that good results can be achieved with as little as 0.05 cc of semen per insemination. Relax pressure on the female's body as soon as possible after insemination so the oviduct can return to its normal position drawing the semen inward.

Points to remember:

1. How often insemination is noticed for satisfactory results varies somewhat among female. It may be best to inseminate more often at the onset of production, but once some eggs have been fertilized, once-a-week insemination is enough to maintain a satisfactory level.
2. Fertile egg can normally be obtained 48 to 96 hours after insemination and up to three weeks thereafter. The percentage of fertile eggs from a flock begins to drop between five and seven days and usually will be unsatisfactory beyond 10 days.
3. Turkeys remain fertile longer than some other birds. Geese show considerable individual variation.

When to consider artificial insemination:

1. To breed from birds of extreme body conformation, for example those with very broad bodies and/ or short legs-conditions which hinder natural mating. The Cornish is an example of this type of bird.
2. To breed from birds with a lot of loose feathering. So you won't have to trim the feathers e.g. Cochins.
3. To breed from injured birds, for example those whose toes have been frozen or wings or legs broken etc.

4. To breed from older males when stiffened joints and other maladies of advanced age prevent natural mating.
5. To make better use of equipment. For example, with this method you can use one pen of females and one pen of males to secure several different mating combinations. You can also carry over unused males rather than having one pen for each mating.
6. To mate incompatible individuals.
7. One male can fertile more females than with natural mating.
8. You can initiate fertility in several females at the same time. With natural mating certain females may be unattended for several days.
9. Prevents over-active males from abusing females.

Recommended housing of the rooster:

The male bird can be housed in individuals' cages, but they need to have enough room to be able to crow. A suggested cage size is 45 cm wide, 60 cm deep and 60cm high. The feed and water containers should be hung on the outside of the cage. Male birds respond to the people handling them and a quiet, unhurried approach is necessary with careful handling. During the collection of semen, it is essential that visitors remain outside the shed. This will prevent the birds from becoming frightened. It is a good idea that the males are housed in close-proximity to the hens. So that the time between collection and insemination is kept to a minimum. Prior to use, the selected male birds should be examined for external parasites, particularly poultry lice and treated accordingly. It is also a good idea to clip the feathers from around the vent area to give easy access to the male organ. This applies particularly to loose feathered breed of poultry.

Semen collection:

For this operation two people are needed, one for holding and collecting the semen (holder), the other (operator) to stimulate the control flow of semen. The holder rests the male birds keel on the palm of this right hand in a horizontal position so that the head is between the holder's side and elbow, the birds' legs being free to move. It is important to hold the male bird loosely to gain the desired result. The holder's left hand is used to collect the semen. The operator holds the roosters' legs loosely but firmly in his right hand and strokes the back of the bird from neck to tall with his left hand. The stroke is firm but not tight and the fingers and thumb follow the laterals contours of the body. After a few strokes, the male organ swells and protrudes outwards and downwards. The white semen will be seen in the central furrow of the organ. The semen is milked down by firm finger pressure either side of the vent into the collecting tube. The male bird should be milked three or four times before insemination is required to check semen quantity and color. If the male bird refuses to produce semen after 10 days of handling or if the semen, which should be white, is discolored due to contamination by fecal material or blood then it is probably useless to preserve with him.

Ejaculate quality and sperm characteristics:

There is a remarkable variation in ejaculate volume and sperm concentration and quality among avian species, even those species of similar size. For example, the American kestrel produces a diminutive ejaculate volume (10-15 ml), containing less than 0.03 to 106 sperm/ ml (compared to the domestic turkey ejaculate that average 300-400ml, containing 8 to 109 sperm/ml). The method of collection and the frequency of natural mating influence ejaculate volume. Although semen quality is low early and late in the breeding season, limited volume also can occur during peak breeding season due to frequent copulations. Larger semen volumes have been associated with males in a pair experiencing compromised fertility, in contrast to highly fecund pairs where the male is ejaculating a smaller volume. Collection attempts earlier in the morning also can improve semen yields and therefore, is standard protocol for domestic turkey stud farms as well as for some wild species (e.g. piping guan). Interspecies difference in sperm morphology, mitochondria numbers, metabolism, motility and duration of storage in the female also are important. These variations, in turn, markedly influence strategies for AI and liquid semen storage/ cryopreservation. Sperm morphology varies from a simple sauropsid form (e.g. domestic poultry) to a complex helical type with an exterior ribbon-like membrane and long flagellum (e.g. passerine) to a rounded, flattened shape (e.g. American kestrel). Avian sperm size (head to flagellum tip length) ranges from 30 to 300 mm and is unrelated to bird mass. In fresh semen samples, sperm pleomorphisms are uncommon, although one study of Houbara bustards revealed that as many as 64% of sperm had large nuclei, perhaps due to aberrant spermatogenesis. The mid-piece appears to be the most variable component of avian sperm, especially in numbers of mitochondria that provide the energy for cellular motility. Although similar in ultra-structural appearance, Japanese quail spermatozoa contain more than 1400 mitochondria, compared to only 20-30 for the turkey. Fowl and turkey spermatozoa have similar morphology and mitochondrial members, yet the former are capable of aerobic glycolysis whereas latter depend on aerobic oxidation. This suggests that there may be variations in energy requirements for sperm motility or survival within the female reproductive tract.

Female reproductive features of interest:

The avian reproductive tract is inherently complex (reviewed in), and this review focuses strictly on these aspects having the potential of influencing AI success. Birds commonly present only a single, functional ovary and oviduct on the left side of the body. As a result, the AI procedure needs to be directed to the left side of the cloaca. The oviduct is comprised of five morphologically distinguishable regions, infundibulum, magnum, isthmus, shell gland and vagina. Successful AI requires an operator who is highly familiar with the specific anatomical features of the species of interest, including having the skill to locate the vaginal orifice. Both the ovary and oviduct size and weight increase markedly as the reproductive season approaches, largely as a result of increased steroidogenesis. Simultaneously, there are enhanced size, vascularization and mucus production in the vagina, development of the brood patch (an area on the chest and abdomen comprised of the feather free skin where the subcutaneous tissue is modified for improved egg surface contact-incubation), abdominal distension and increased flexibility and distance between

the public bones, all being collective indicators of an ideal time to AI, it must be emphasized that the oviduct is susceptible to disease. Insemination must be performed carefully. If not one can expose the vagina to infection, trauma or stress that in turn can cause egg yolk peritonitis (i.e. yolk entering the abdominal cavity) or hyper peristalsis that can elicit premature laying of soft shelled eggs. All domestic and wild birds examined to date have at the anterior end of the vagina, a utero-vaginal junction (UVJ) and specialized tubular invaginations of the surface epithelium known as sperm storage tubules (SST). This important adaptation no doubt improves overall reproductive fitness by ensuring the presence of sperm, potentially important in the case of a wild female losing its mate, thereby allowing a recultch. In an ex situ breeding program, the SST serves as a sperm reservoir to facilitate fertilization between inseminations. Although the length of sperm storage is unknown for most species, fertile eggs have been produced as long as 45 days after AI in the domestic turkey. Typically 6-10 days intervals between successive inseminations are used in commercial poultry industry programs to ensure fertility. The SST also has been observed microscopically in the folds of the mucosa of the American kestrel, Peregrine falcon, European eagle owl and Marsh harrier. Although the physiochemical mechanism for sperm storage and release has not been well studied for birds, the presence of the SST offers a unique advantage for avian AI programs by extending the window of opportunity for fertilization and reducing the number of required insemination. This is a fascinating and rich ares for future research.

Insemination of the hen:

The hen is held by the left hand being placed over the breast with the birds back forced against the holders' body, the head pointing to the ground. The right hand is placed over the vent so that the thumb is above and forefinger below the vent. A sudden pressure exerted around the breast area and at the same time using the thumb and forefinger to spread apart the cloaca, resulting a in the turning of the cloaca inside out. The operator, with 0.1 ml of semen or 0.02 ml of diluted semen (in PBS or saline) drawn up in the inseminating tube, places this tube as far as possible in the exposed oviduct opening seen at the left side of the intestinal opening. The menen is introduced at the semen time as the holder release the pressure and the cloaca returns to its normal position. Usually the semen from 1 rooster can be used for 40-50 hens.

Regularity of insemination:

Insemination should be carried out on two consecutive days the first week and then once each week thereafter while fertile eggs are required. As poultry semen has a very limited life, insemination of hens should be complete within one hour of semen collection. It is a good idea to carry out the operation at the same time each day, the best time being between 2.00 pm and 4.00 pm. The reason for this is that during the morning, most hens have an eggs in the oviduct, thus obstructing the free passage of semen to ovary. Another point in favor of inseminating the hens in the afternoon is that it is generally cooler and the hens are less likely to be affected by heat, particularly in late spring. Observation has shown that eggs are fertile after the second day of insemination and can remain fertile for two weeks or more. If another male is to be used on the

same hen in a breeding program, it is suggested that a period of three weeks elapse before the second male is used. If large numbers of male birds are to be used for artificial insemination, it is suggested that, prior to their use, a sample of the semen he examined under a microscope to check sperm motility as them is a good correlation between sperm movement and fertility.

Management and selection of AI candidates:

We have found that certain procedures maximize fertility. First, it is prudent to consider fasting the female 24 hrs. in advance of a scheduled AI, largely to minimize the presence of accumulated urates and fecal debris in the urodeum. Second, it is important to capture and handle the bird in a physical area away from the nest to avoid accidental egg breakage. Third, insemination should be conducted in birds that have had the opportunity to naturally mate or express a copulatory display as both activities facilitate sperm transport post-AI. Finally, all females (regardless of type) are rewarded with a favorite food immediately after sperm deposition.

Artificial insemination timing:

In general, larger size avian species (e.g. eagles and cranes) are inseminated twice per week during the 2 week prior to onset of egg laying and then after each oviposition. In contrast, smaller counterparts (e.g. Booted eagle or Eleonoras falcon) are inseminated three times during the week before oviposition begins. Of course optimal AI protocols are species dependent and related to specific biological norms. The three most critical factors are most practical insemination method required minimum sperm number and duration of female fertility for the species of interest. For larger species laying only one or two eggs per breeding season, it is advisable to start insemination 7-14 days in advance of the first oviposition. For example optimal fertility is achieved in cranes inseminated 10-14 days before the first egg is laid. Some birds easily tolerate twice per week inseminations for two or three consecutive weeks in advance and few hours after each oviposition. Two or three inseminations every other day or every 3 day also has produced excellent results in pheasants. Japanese quail often are inseminated every other day whereas good fertility occurs in turkey by inseminating only once every 3 week. For species or individuals prone to stress, it is prudent to inseminate only once on the day before the first oviposition, thereby sacrificing the first egg to secure fertility in the second and subsequent eggs. Otherwise, such sensitive females may refuse to lay or oviposition is delayed in the face of multiple restraints. However, once laying begins, egg production rarely is interrupted by insemination, regardless of bird type. In general the smaller the body mass, the shorter the duration of fertility. Additionally it appears that sperm retention in the SST is improved by inseminating before the first egg is laid. Recent endocrine monitoring data from our laboratory also suggest that there are hormonal profile traits during a few days prior to the first oviposition that also signal a milieu highly accommodating to sperm, a period when the female readily accepts copulation at a higher frequency. Studies in the turkey have demonstrated that delivering sperm immediately before egg production onset maximizes sperm filling the SST, thereby promoting fertility throughout the laying interval. It also is clear that AI should be performed as soon as possible after each oviposition. However the oviductal expulsion

peristalsis associated with laying continues for at least 30 min, which compromises the ascendant passive transport of the deposited sperm. Therefore, AI should occur coincident with the antiperistalsis associated with retraction of the uterus. In contrast, AI immediately prior to egg release has been related to decrease fertility, perhaps because fewer sperm reach the SST or more are being expelled during egg laying. The actual practicality of inseminating soon after each egg is laid depends on the minimum time required between AI and egg laying that, in turn, is species specific. For example, this interval is 60 hours in the peregrine falcon. Thus, for such females that lay an egg every other day, a given insemination will fertilize the third egg in a sequence, but not the one produced on the next day after AI. Insemination efficiency overall also may be influenced by time within the breeding season. The suggestion that there is incomplete filling of the SST early during egg production followed by ineffective storage later in the season has been refuted by Robinson et al. Likewise, there has been at least one report suggesting that Golden eagle eggs laid later in sequence are more fertile than earlier counterparts. Finally it has been asserted that older females probably require more insemination as aging adversely influences SST integrity or function.

Depth of insemination:

Although much more effective than cloacal insemination, vaginal AI remains highly inefficient. For example, in the Bengalese finch, 95-99%mof deposited semen leaks almost immediately from the vagina and is lost. Therefore deep intravaginal AI is the best approach for achieving high fertility, as long as depth of insertion does not completely by-pass the uterovaginal SST. Lengths of insertion compatible with the intramagnal AI technique should be restricted to special circumstances where only a few spermatozoa are available and insemination is planned for a known time of ovulation.

Depth of insemination depends largely on species size and the presence/ absence of fully formed eggs. For example in the case of the latter, length of insertion in the Bonelli's or Golden eagle decreases from 35 to 25 mm. at times prior to onset of egg laying the level of female receptivity and endocrine status significantly influence depth of cannula insertion. Additionally if the oviduct has not been everted the cannula requires deeper penetration to compensate for the absence of oviductal protrusion.

Equipment's required:

The equipment need not be lavish or expensive. It consists of a glass or plastic test tube for collecting semen from the male a 3cc hypodermic syringe with 0.1ml graduations a rubber connection and a 0.5cm external diameter glass inseminating tube 9cm in length. Sometimes a small plastic funnel is used where semen collection may be difficult. Artificial insemination equipment is simple. Commercial operators may use more complicated equipment, including injection guns, collection aspirators and temperature controlled semen containers.

Using the diluents:

In order to increase the number of hens that can be inseminated from the same rooster, the semen may be diluted with a solution known as modified Ringer's solution.

Composition of diluent:

Sodium chloride	68 gm
Potassium chloride	17.33 gm
Calcium chloride	6.42 gm
Magnesium sulphate	2.50 gm
Sodium bicarbonate	24.50 gm
Distilled water	10000 cc

In turkeys

Cryopreservation of poultry semen:

The effect on fertility of storing the semen of fowl, turkey, gander or drake at -196 degree C in a diluent with di-methyl-acetamide was investigated. After artificially inseminating ducks, geese, turkeys and hens with freeze-thawed semen, the percentage of fertility was 69-90%, 81-90%, 80-90% and 75-85% respectively.

Chapter 7
Reproductive Biotechnology

Biotechnology: It may be defined as "any advanced technique that generally used living organisms to make or modify a product, to improve plants or animals or to developed micro-organism for specific genetic proposes".
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It mainly focuses on the development and application of modern bio-technology based on new enabling techniques of recombinant DAN technology as well as genetic engineering.

Biotechnology has great potential to influence and benefit agriculture, forestry, fisheries and livestock. Advanced and modern technique of biotechnology offer the potential of moving any cloned gene from any organism into any other organism and confer much greater precision and speed in achieving results than conventional techniques.

Assisted Reproductive Technologies

1. AI
2. In vitro maturation of oocyte
3. In vitro fertilization and culture of oocyte
4. Embryo transfer technologies
5. Oocyte and embryo preservation
6. Genetic engineering and cloning
7. Separation of X or Y spermatozoa
8. in vitro development of small follicle
9. Ovum pick-up (OPU)
10. Semen evaluation
11. others

Modern biotechnology holds promise of:

- Increased and sustainable productivity
- efficient processing for improved product diversification
- utilization & adaptation of product quality to functional requirements
- decreased reliance on agrochemicals and other external inputs
- it promotes better conservation and use of genetic resources and
- environmentally friendly natural resources management can be assured

Biotechnologies are already being used to elevate a new strains of crop plants, new plant and animal diagnostic products, animal vaccines, biological pesticides and herbicide other biological control agents and modification and improvement of domestic animals used for food production.

The possible uses of biotechnology in the field of livestock development:

1. Production of transgenic animal
2. Wide use of monoclonal antibodies for efficient diagnosis leading to safe and specific treatments for animals diseases
3. Production of vaccines for the prevention of viral, bacterial and parasitic animal diseases
4. Production of pathogen-specific vaccines
5. Production of endocrine-directed vaccines for stimulation of training in beef cattle.
6. Production of vaccines that compensate for various stress-induced production losses.

Use of substances: Produced by biotechnology methods to improve the productivity of farm Animals. Example may be growth hormone injections.

Porcine growth hormone (PST) may be used in moderate dose for pig-higher doses have detrimental effect on growth performance. In elderly humans growth hormone injection increase lean caresses in livestock.

Bovine growth hormone (BST) will increase milk production in dairy cows.

Biotechnology could really improve animal welfare through the development of better animal vaccines

Transgenic farm Animal: Where a gene from another species is incorporated into an animal. In 1982 R.D. Palmiter and his colleagues inserted rat growth genes into mice and created a giant mice, in 1983 they created giant mice by inserting human growth genes. The human growth gene was heritable in the standard Mendellian manner. Researcher at the University of Edinburgh have now succeed in producing transgenic lambs which can also pass human gene on to their offering. Genes also could be introduced into farm animals to produce disease resistance. An example would be the gene for tick resistance. It could be transferred from zebu to European cattle. The most radical transgenic idea is to replace the mitochondria in cattle skin cells with chloroplasts. Chloroplasts are the photosynthesis cells of plants. Chloroplasts are the photosynthesis cells of plants. Chloroplasts would enable cattle to eat less food because they could derive a portion of their energy from sunlight.

It is more likely that the creation of transgenic animals has a greater potential for creating welfare problems compared to use products derived from technology such as vaccines and hormones.

Animal pharmaceutical Factories: Another use for transgenic animals is producing medically valuable substances such as clotting factor for hemophiliacs. These substances would be produced in the milk and could be continually harvested via milking. Institute of Animal physiology in England have produced sheep which produced medically useful clotting factor for hemophiliacs. The ability to produce the anti clotting factor can be passed on to the offspring with the procedure used for production of transgenic animals.

Creating to Mosaic animals: Genetic material from two different species are combined to form an animal which is a genetic mosaic. Sheep and goat genes will produce an odd looking animal which has patches of goat tissue and patches of sheep tissue throughout its body. Wool grows out of one body area and goat hair out of another. It is named as geep (goat-sheep mosaic) at the University of California.

Insertions of genes: Is the inserting genes into animals which will enable them to make medically valuable substances in their milk.

Adding and deleting genes: The latest developments in biotechnology enable researchers to inactivate specific genes in mice. This method will enable scientists to probe the purpose of a single gene throughout the life of an animal. This research has tremendous potential to improve the welfare of both people and animal by finding cures for genetic disorders.

Indirect uses of Biotechnology: Products developed using the tools

i. Biotechnology can also be used as diagnostic tests for diseases or for identification. DNA fingerprint can be used to detect stolen animals and their meat and this could eliminate painful branding.
ii. DNA fingerprinting could also be used to assist law enforcement officials on determining the origin of products made from poached wildlife.
iii. Biotechnology can also be used to develop faster and more accurate diagnostic tests for animal diseases.

Ideas for future improvement on the efficiency of animal Agriculture

1. Modify animals genetically so that they no longer are seasonal breeders.
2. Add double muscling and growth genes to the Y-chromosome.
3. Develop in vitro oogenesis and spermatogenesis. This results in unlimited gametes for breeding purposes except for gestation.
4. Increase appetite to get more production in relation to maintenance costs
5. Develop methods or genes for hibernation to over winter animals.
6. Modify animals genetically for earlier puberty and markedly shorter gestation.
7. Control timing and rate of ovulation precisely to produce twins in cattle, sheep and goats, large litters in swine and daily ovulation in chicken.

8. Karyotype and test embryos for enzyme deficiencies before embryo transfer. This may result in markedly less embryonic death & obviates (prevent) the need to sex of semen.
9. Rejuvenate hens by stimulating molting.
10. Add genes for generalized diseases resistance
11. Decrease turnover of gut epithelium to markedly decrease maintenance costs.
12. Develop high protein milk, low fat meat and low cholesterol eggs.
13. Dispense with difficult births by having young born at much smaller sizes.
14. Exploit mitochondrial and other cytoplasm inheritance
15. Improve biotechnical techniques such as cryopreservation, pregnancy tests, artificial insemination, in vitro fertilization, embryo transfer, gene transfer, cloning, gene detection and mapping, genetic treatment of different diseases, major gene detection etc.

Estrus Synchronization

Synchronization of estrus: Synchronization of estrus is a procedure by which a significant number of female animals in a herd brought into estrus at predetermined time.

Principles

1) By removing or inducing the dermis of the corpus luteum (CL). So that all animals in an appropriate group enter the follicular phase of the cycle at the same time and hopefully synchronized at the ensuring estrus.
2) Approach involves suppression of follicular development during an artificially extended lineal phase so that upon removal of pharmaceutical blockade after a sufficient period of treatment, all animals should enter the follicular phase approximately synchronously.

General ways of controlling the life span of CL:

There are two general ways of controlling the life span of CL and the subsequent onset of estrous and ovulation.

i. The first method of controlling the span of CL is to administer a luteolytic agent that shortens the natural life span of the CL. When the luteolytic agent is given, CL regression usually occurs within 24 to 72 hours and estrus and ovulation follow within 2 to 3 days.
ii. The 2nd method involves long-term administration of a progestogen so that the CL regresses naturally during the period when progestogen is being administered. This exogenous progestogen continues to exert negative feedback on LH production after CL regression has occurred. After withdrawal of progestogen at any time, follicular growth, estrus and ovulation occurred within about 2 to 8 days. Generally long term progestogen treatment is characterized by administering the progestogen for 14 to 24 days.

In all species, the CL is responsive to luteolytic agents only during certain stages of its development.

- In ruminant and horse non-responsive period is 4 to 6 days of the cycle.

- ❖ In pig it is 12 to 13 days.

The two primary luteolytic agents are:

- ❖ Estrogen- only in ruminants
- ❖ Prostaglandin $F_{2\alpha}$- all species

Methods of synchronization

1. Luteal enucleation
2. Injections of progesterone
3. Orally active progestagens
4. Silastic implants
5. Intra-vaginal sponges
6. Short term progestagens
7. Prostaglandin injection

Estrogen + Progestogen : Injection on day 1 with progestogen implants for 9 days beginning on day 1 and A.I at estrus or fixed time. Majority bred within 3 to 5 days period. Repeat cycles are also synchronized.

Progestogen + Prostaglandin : Progestogen for 7 days with PGF given on day 6. A.I. at estrus or fixed time. Majority bred within 2 to 3 days period repeat cycles are also synchronized.

Sheep

1. Intra-vaginal sponges
2. Short term progestogen
3. Prostaglandin injection
4. Progestogen + PMSG = progestogen for 12 to 14 days, PMSG given at progestogen withdrawal. Majority bred within a 2 days period.

- Injection of progesterone
- Orally –active progestogens
- Non- steroidal pituitary blockade
- Progestaglandin injection
- Gonadotrophin injections

Horse:

1. Progestogen for 15 days, majority bred within a 4 to 7 days period.
2. Prostaglandin: one dose in diestrus , majority bred within a 3 to 5 days period.
3. Prostaglandin + HCG on day 21 or 22. Majority bred within a 2 to 4 days period.

Superovulation and synchronization of estrus in goats:

Goat:

1. Progestogen + PMSG: Progestogen for 18 to 12 days apart, majority bred within a 2 to 3 day period.
2. Prostaglandin: Give 2 injection 11 to 12 days apart, majority bred within a 2 to 3 days period.

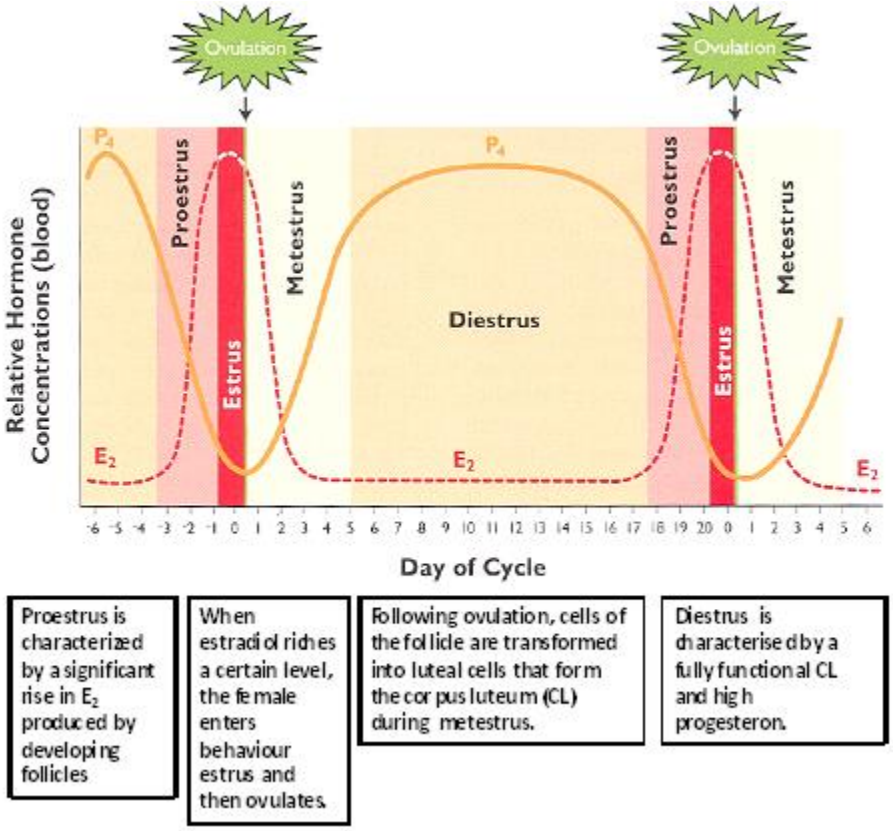
Drug	Doses and administration	Given in association with
MAP	60mg; intravaginal sponge removal 14 days	500 iu im PMSG injection. Day of sponge withdrawal
FGA	30-40mg; intravaginal sponge removal 12 days	500iu PMSG + 25mg PGF2α
$PGF_{2\alpha}$	16mg; 2-8 mg im injection 11 days apart	
PMSG	1500-1000 iu; im single injection last days progesterone treatment	17-22 days progesterone inj. 12 mg/ days ism
PMSG	500-1000 iu; im injection	500 iu of HCG
FSH+ $PGF_{2\alpha}$	125 mg cloprostenol im inj at sponge insertion 9 days later start FSH twice daily	MAP 60 mg intravaginal sponge for 11 days

Synthetic progestogens:

- Norgestomet
- Medroxyacetate progestogen (MAP)
- Melengestrol acetate
- Fluorogestone acetate (FGA)
- Altrenogest

Estradiol:

- Estradiol valerate
- Estradiol benzoate
- Estradiol cypionate



Described the methods of synchronization:

1. Luteal enucleation: removal of the sources of progesterone by enucleation of the CL by digital pressure per rectum.
2. Injection of Progesterone: Suppression of estrus is achieved when an appropriate dose of progesterone is administered such as 50mg per day- repeated injection for 18-20 days.
3. Orally- active progestogen:
    - Melengestrol acelate (MGA)
    - Medroxy progesterone acetate (MPA)

    Feeding for 15-18 days- estrus exibit 3-5 days after withdrawal.

4. Silastic implants: Silastic implants containing MGA were inserted subcutaneously in the neck region of cows for 22 to 64 days and upon withdrawal of the implants, 64% of 45 cows were in estrous 36-72 hours later.
5. Intra-vaginal sponges: Sponges are soaked in oil containing the progestagens, dusted with an antibiotic preparation, Positioned deep in the vagina and left for a period of 18-21 days. Following withdrawal of the sponges, estrus noted within 24 to 72 hours.

6. Short term progestagens: The use of a syntheyic progestagen administered in implants, sponge passaries or coils for a period of only 9-10 days, in conjunction with an injection of 5-7.5 mg oestradiol benzoate and 50-250 mg progesterone given at the onset of treatment.

Potential advantages synchronization of estrus

- To reduce time needed for detection of estrus in a group of animals.
- To facilitate the use of A.I. by treating animals in groups.
- In conjunction with a procedure for controlling the time of ovulation, to permit insemination on a predetermined schedule.
- To enable the feeding of animals in uniform groups.
- To limit the overall period of parturition in a herd or flock.
- To permit supervision at birth, in order to reduce neonatal mortality and arrange cross fostering.
- It permits weaning, fattening and marketing of uniform groups of animals.
- To enable sticker measure for the control of disease.
- Facilitate the use of embryo transplantation.
- Rationalize the use of labor, buildings and others resources in every step.

Potential tool for future research and livestock development

Multiple ovulation and embryo transfer (MOET)

Embryo transfer refers to the techniques by which fertilized ova are collected from a female called the donor and transferred, for development to term to another female known as the recipient.

Transplantation of embryo:

- ✓ The first recorded transfer of mammalian eggs was in 1891 by Heapa
- ✓ Pincus in 1930
- ✓ More extensive application by Hammond's research group in the late 1940s
- ✓ In man- 1978

Biological flexibility:

1) The lumen of fallopian tube and uterus represents an immunologically privileged site to the extent that embryonic and placental tissues bearing foreign antigens are not normally rejected within a species, putting them in appropriate time.
2) The mammalian embryo exists for a variable period in a free-living state in the lumen of the uterus before the process of implantation or attachment is initiated.

Steps for MOET:

1. Selection and management of Experimental animals.
2. Treatment of donor and recipient animals.

3. Superovulation of donor animals.
4. Synchronization of recipient animals.
5. Insemination of donor animals.
6. Collection of embryos from the donor animals.
7. Grading of embryos.
8. Transfer of embryos to the recipient animals.
9. Pregnancy detection.
10. Data collection.
11. Statistical analysis.

Examples of some doses of PMSG and HCG commonly administered to induce superovulation.

Species	Doses of PMSG injected iu/ intramuscularly	Doses of HCG injected intramuscularly/ intravenously
Cow	2000-3000	500-2000
Sheep	500-800	250-500
pig	750-1500	500-1000

Examples of various treatments used for inducing ovulation in animals:

Species	Age (month)	Nature of treatment
Calf	1.5 – 3	2000 iu PMSG + 2000-10000 iu HCG 5 days after
	2-4	1500-2000 iu PMSG + 1500 iu HCG 5 days later
	3	1800 iu PMSG and progestogen blockade for 4days
Lamb	1-5	100 iu PMSG + 500 iu HCG 4 days after
	2	350-700 iu PMSG + 500 iu HCG 2-3 days later
Pig	3-4	500-1250 iu PMSG + 500 iu HCG 4 days later
	5	250-2000 iu PMSG + 500 iu HCG 2 days later

Doses of Gonadotropins for superovulation

Animal	Days of estrous cycle	Gonadotropin for follicular growth		Gonadotropin for ovulation	
		PMSG (iu)	FSH (mg)	HCG (iu)	LH (mg)
Cow	15-16	1500-3000	20-50	1500-2000	75-100
Calf	-	1000-2000	20-50	1000-1500	50-75
Gant	16-17	1000-1500	12-20	100-1500	50-75
Kid	-	1000-1200	10-15	1000-1500	50-75

Ewe	12-14	1000-2000	12-20	1000-1500	50-75
Lamb	-	1000-1200	10-15	1000-1500	50-75
Pig	15-16	750-1500	10-20	500-1000	25-50
Rabbit	-	25-75	2-3	25-75	2-30

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IN VITRO PRODUCTION OF EMBRYOS (IVP)

1. Collection and processing of ovaries

1.1. Preparation for ovary collection

Physiological saline of 0.9% NaCl was prepared for washing of ovaries. The saline solution was sterilized in autoclave and stored in a refrigerator for further use. On the day of collection, 1000 mg of zentamycin were added per liter of saline solution. The solution was warmed at 25^0C to 30^0C and kept in a thermos box to maintain this temperature during transporting the ovaries from slaughterhouse to the laboratory. Dulbecco's Phosphate Buffered Saline (D-PBS) solution was also prepared by adding one pack of PBS salt (Sigma Chemical Co., USA) in one liter of distilled water. Then it was sterilized in autoclave and stored in a refrigerator for further use.

1.2. Collection of ovaries and trimming

The ovaries were kept in collection vial containing 0.9% physiological saline in a thermo flask at 25^0C to 30^0C and transported to the laboratory within 4 to 5 hours of slaughter. The ovaries were then transferred to the sterilized petridishes containing same saline. The ovaries were rinsed thoroughly by physiological saline solution at 25^0C and recorded for the presence or absence of corpus luteum (CL). In the laboratory each ovary was trimmed to remove the surrounding tissues and overlying bursa.

2. Evaluation of ovary

After collection and trimming, ovaries were evaluated on the basis of CL presence (A) and absence (B)

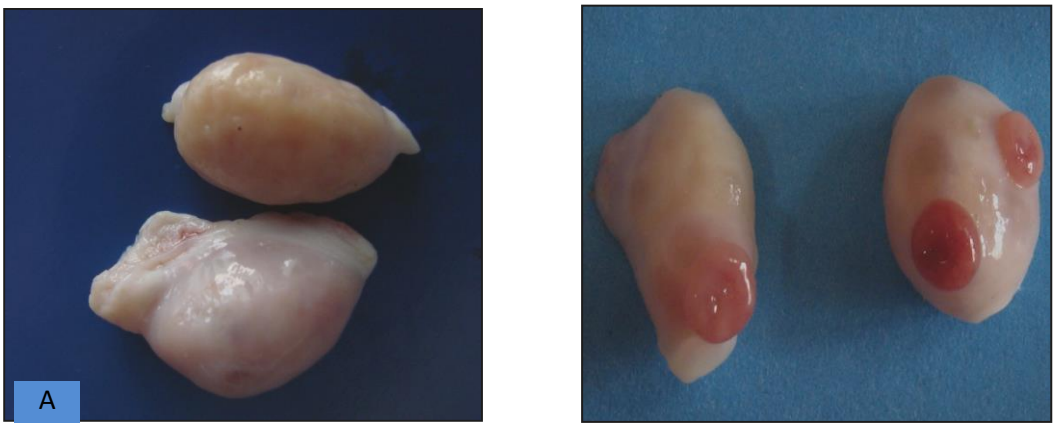


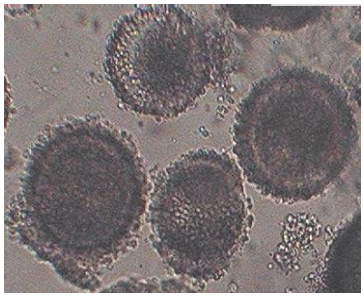
Figure 1.Representative photograph showing

A= Ovaries without corpus luteum;
B= Ovaries with corpus luteum (arrow indicates the CL)

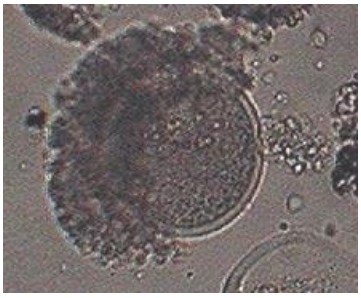
3. Collection of oocyte and evaluation of COCs

Aspiration: The 10 ml syringe was loaded with D-PBS (1.0-1.5ml), and the needle (18 G) was put in the ovarian parenchyma near the vesicular follicles and all 2-6 mm diameter follicles were aspirated. After aspirating the follicles from one ovary, it was precipitated for 10 minutes (Nolasco *et al.*, 2013) then the upper part was removed and the lower part was entered into a 90 mm petridish, avoiding damage to the cumulus cells as shown in fig 1.

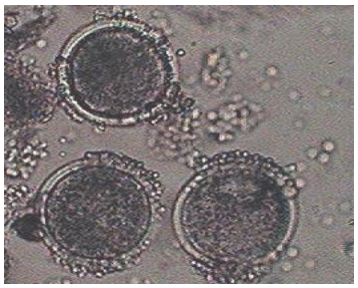
The COCs were searched and graded under microscope at 10x magnification. The COCs were classified into 4 grades on the basis of cumulus cells and nucleus as described by Khandoker *et al.* (2001). The grades were; Grade A: oocytes completely surrounded by cumulus cells; Grade B: oocytes partially surrounded by cumulus cells; Grade C: oocytes not surrounded by cumulus cells and Grade D: degeneration observed both in oocytes and cumulus cells.



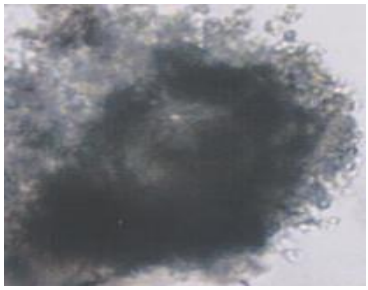
Grade A



Grade B



Grade C



Grade D

Representative photograph showing different grades of cumulus-oocyte-complexes

Grade-A= COCs completely surrounded by cumulus cells,

Grade-B= COCs partially surrounded by cumulus cells,

Grade-C= Oocytes not surrounded by cumulus cell and

Grade-D= Degeneration observed both in cumulus cells and oocytes.

Grade A and Grade B considered as Normal COCs and taken for further study
Grade C and Grade D considered as Abnormal COCs and discarded

4. *In vitro* maturation of COCs

The maturation medium, TCM-199 (Sigma Chemical Co., USA) was prepared and the pH of all media was adjusted to 7.4 on the day of oocyte collection and sterilized by passage through a 20 µm filter. About 2.5-3.5 ml of the medium was poured into 35 mm culture dishes. This culture dishes were used for washing of COCs then 1-4 drops (depending on number of oocytes) of 100 µl of medium were poured in another culture dish and the COCs were transferred to the droplets and covered with paraffin oil. Finally, the dishes with droplet were kept in an incubator at 38.5^0 C

with 5% CO_2 in humidified air .After 24 hours culture of COCs in maturation medium, the level of nuclear maturation was checked.

5. *In vitro* fertilization

Medium preparation

The fertilization medium, Brackett and Oliphant (BO) was prepared and its pH was adjusted to 7.8 on the day of use. Finally it was sterilized by passing through a 20 µm Sartorius Minisart filter.

Semen collection

Semen was collected by artificial vagina (AV) method from the bucks and brought to the laboratory in icebox (at 4-5°C) within 4-5 hours.

Semen preparation (sperm capacitation)

The sperm concentration of raw semen was calculated by haemocytometer. Fifty µl of raw semen was taken in 10 ml sterilized pipette and 3.0-4.2 ml (depending on the sperm concentration) of semen washing solution was added to adjust the sperm concentration to $2x10^6$ per ml. Then the semen with washing solution was taken in a centrifuge tube and it was centrifuged at 800 rpm for 5 minutes at 30^0C. After 5 minutes, the top liquid portion was removed by the digital pipette. Then same amount of semen washing solution was added to the centrifuge tube. The same procedure was repeated twice and finally the sperm concentration was adjusted at 10^6 per ml by adding semen dilution solution. Then 1-4 insemination droplets (100 µl) of Brackett and Oliphant medium depending on the number of the matured COCs in a 35 mm culture dish were prepared, covered with paraffin oil and were kept in the incubator for 3-4 hours for preincubation.

Insemination (incubation with sperm)

After 24 hours of maturation, the half of the matured COCs was proceed to fertilization and other half was used for nuclear maturation test. Two 35 mm culture dishes were filled with COCs washing solution and the COCs were washed 3 times. About 15-20 COCs with minimum volume of medium were transferred to each of the sperm drops prepared previously and then incubated for 5 hours in incubator at 38.5^0C with 5% of CO_2 in humidified air.

Evaluation the fertilization rate

After 5 hours of incubation, all the COCs from each drop were denuded from cumulus cells by repeated pipetting.

a) Oocyte with two PN – normal fertilization
b) Oocyte with one PN – asynchronous PN development/parthenogenetic activation or one PN was obscured by lipid droplets
c) Oocyte with more than two PN – polyspermia

***In vitro* culture and observation of embryo development**

After 5 hours incubation, the fertilized ova were taken from the semen drops with cumulus cells by using the appropriate micropipette. Then they were washed three times in pre-incubated medium (TCM-199) and were transferred to other culture drop (100 μl) of TCM-199. The dish was then kept in the CO_2 incubator at 38.5^0C with 5% of CO_2 in air . The development rate was checked every 48 hours and the culture were continued for 6 to 7 days. Finally the numbers of 2-cell, 8-cell and compact morula were recorded.

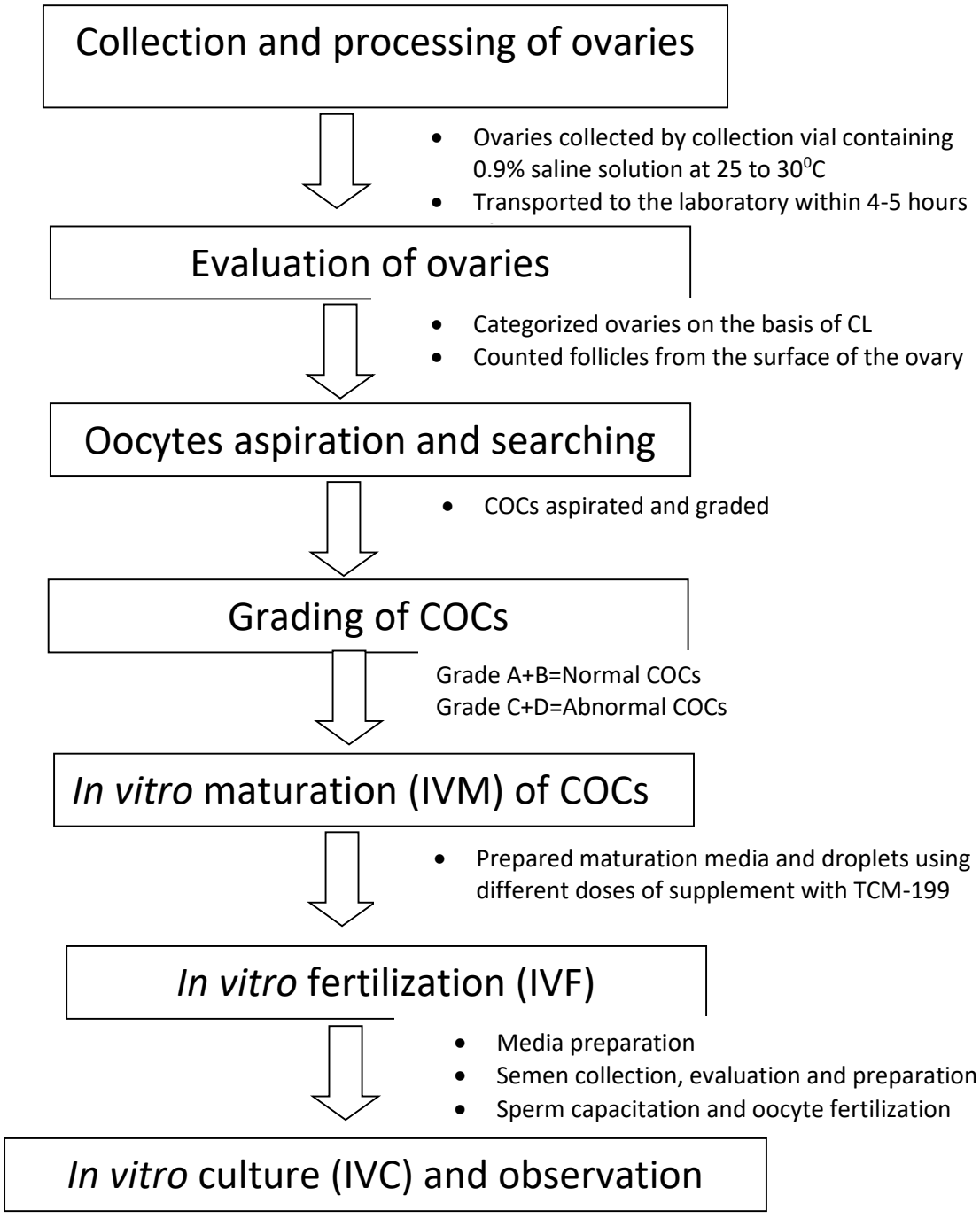


Figure 3.1. Flow diagram of collection, evaluation, *in vitro* maturation, fertilization and culture of goat oocytes

Cryopreservation

Cryobiology is the branch of biology that studies the effects of low temperatures on living things. The word cryobiology is derived from the Greek words "cryo" cold, "bios" life and "logos" science. In practice, cryobiology is the study of biological material or systems at temperatures below normal. Materials or systems studied may include proteins, cell, tissues, organ or whole organisms. Temperatures may range from moderately hypothermic conditions to cryogenic temperatures.

Cryopreservation: cryopreservation is a technology whereby cells, whole tissues or embryos are preserved by cooling to temperatures below the freezing point of water.

Major areas of study in cryopreservation:

6 major areas of study in cryobiology can be identified

1. Study of cold-adaptation of micro-organisms, plants animals.
2. Cryopreservation of cells, tissues, gametes and embryos.
3. Preservation of organs under hypothermic conditions for transplantation.
4. Lyophilization (freeze-drying) of pharmaceuticals.
5. Cryosurgery
6. Physics of super cooling ice nucleation or growth and mechanical engineering aspects of heat transfer during cooling and warming.

Basic principles of cryobiology

1) Due to resiliency of living cell, the study of life at reduced temperatures (cryobiology) is possible.
2) The literature of cryobiology is vast and covers everything from the freezing of cells to animal hypothermia. For the purposes of this overview, we will consider only phenomena that occur below the normal freezing point of pure water, which is defined as 0^0c.
3) The phenomena of cryobiology can be thought of as arising from two contradictory effects of temperature reduction, one is a reduction in the rate of deterioration of biological systems and other effect is destructive in nature.
4) The zone of hazardous subzero temperatures depends on the biological system.
5) As the temperature is reduced, less and less energy exists in the system to drive these kinds of molecular motions.
6) At several degrees below the commonly-observed ultralow temperature, molecular motions are so slow that changes are nil for all practical purposes even over time spans of several hundred years or more.
7) When a cell is permeated by cryoprotectants in concentrations high enough to allow vitrification, the entire cell's molecular constituents become locked into the glass as it forms and therefore become unable to change over time.
8) Vitrification prevents damage related to ice formation.

9) Not all damage is avoided by vitrification, however, there is also damage caused by low temperature exposure per second, which is called either chilling injury or cooling injury.

Semen cryopreservation

Semen cryopreservation is a procedure to preserve sperm cells. Semen can be used successfully indefinitely after cryopreservation. For animal sperm, the longest reported successful storage is 21 years. It can be used for sperm donation where the recipient wants the treatment in a different time or place.

Freezing: the most common cryoprotectant used for semen is glycerol (10% in culture medium). Often sucrose or other di-saccharides, tri-saccharides are added to glycerol solution. Semen is frozen (-196^0c) using either a controlled rate, slow –cooling method (slow programmable freezing or SPF is a typical cooling rate around 10c/minute) or a newer flash-freezing process known as vitrification (ice crystal formation).

Thawing: thawing at 40^0c seems to result in optimal sperm motility. On the other hand, the exact thawing temperature seems to have only minor effect on sperm viability.

Refreezing: three cycles of freezing and thawing can be performed to test motility. This is provided that samples are frozen in their original cryoprotectant and are not going through sperm washing or other alteration in between, and provided that they are separated by density gradient centrifugation.

Oocyte cryopreservation: animal oocyte cryopreservation (egg freezing0 is a novel technology in which a female's eggs (oocyte) are extracted, frozen and stored. Later, when she is ready to become pregnant, the eggs can be thawed, fertilized and transferred to the uterus as embryos.

Methods

At first, one to several weeks of hormone injections that stimulates ovaries to ripen multiple eggs. When the eggs are mature, a medication to trigger ovulation is given and the eggs are removed from the body using an ultrasound-guided needle. The procedure is usually conducted under sedation. The eggs are immediately frozen.

The eggs is the largest cell in the animal body and contains a great amount of water. When the egg is frozen, the ice crystals that from can destroy the integrity of the cell. To prevent this, the egg must be dehydrated prior to freezing. This is done using cryoprotectants which replace the water and inhibit the formation of ice crystals.

Eggs (oocytes) are frozen using either a controlled-rate, slow-cooling method or a newer flash-freezing process known as vitrification. The slow-cooling method is the most practiced of embryo

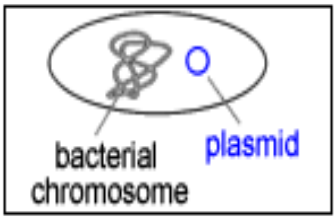
freezing techiques. Virtification is much faster but requires higher concentrations of cryoprotectants to be added. The result of virtrification is a solid glass-like cell, free of ice crystals. Once frozen, shell of the eggs hardens. Thus, currently, when eggs are thawed, a special fertilization procedure is performed by an embryologist whereby sperm is injected directly into the egg with a needle rather than allowing sperm to penetrate naturally by placing it around the egg in a dish. This injection technique is called ICSI (Intracytoplasmic sperm injection) and is also used in IVF.

Cloning

In biology it 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 Greek word for "trunk, branch", referring to the process whereby a new plant can be created from a twig. Cloning an organism means to create a new organism with the same genetic information as an existing one. In a modern context, this can involve somatic cell nuclear transfer in which the nucleus is removed from an egg cell and replaced with a nucleus extracted from a cell of the organism to be cloned (Currently both the egg cell & its transplanted nucleus must be form the same species). As the nucleus contains almost all of the genetic information of a lifeform the host egg cell will develop into an organism genetically identical to the nucleus donor.

Different types of cloning: The following three types of cloning technologies will be discussed: (1) recombinant DNA technology or DNA cloning, (2) reproductive cloning, and (3) therapeutic cloning.

Recombinant DNA Technology or DNA Cloning



The terms "recombinant DNA technology," "DNA cloning," "molecular cloning," and "gene cloning" all refer to the same process: the transfer of a DNA fragment of interest from one

organism to a self-replicating genetic element such as a bacterial plasmid. The DNA of interest can then be propagated in a foreign host cell. This technology has been around since the 1970s, and it has become a common practice in molecular biology labs today.

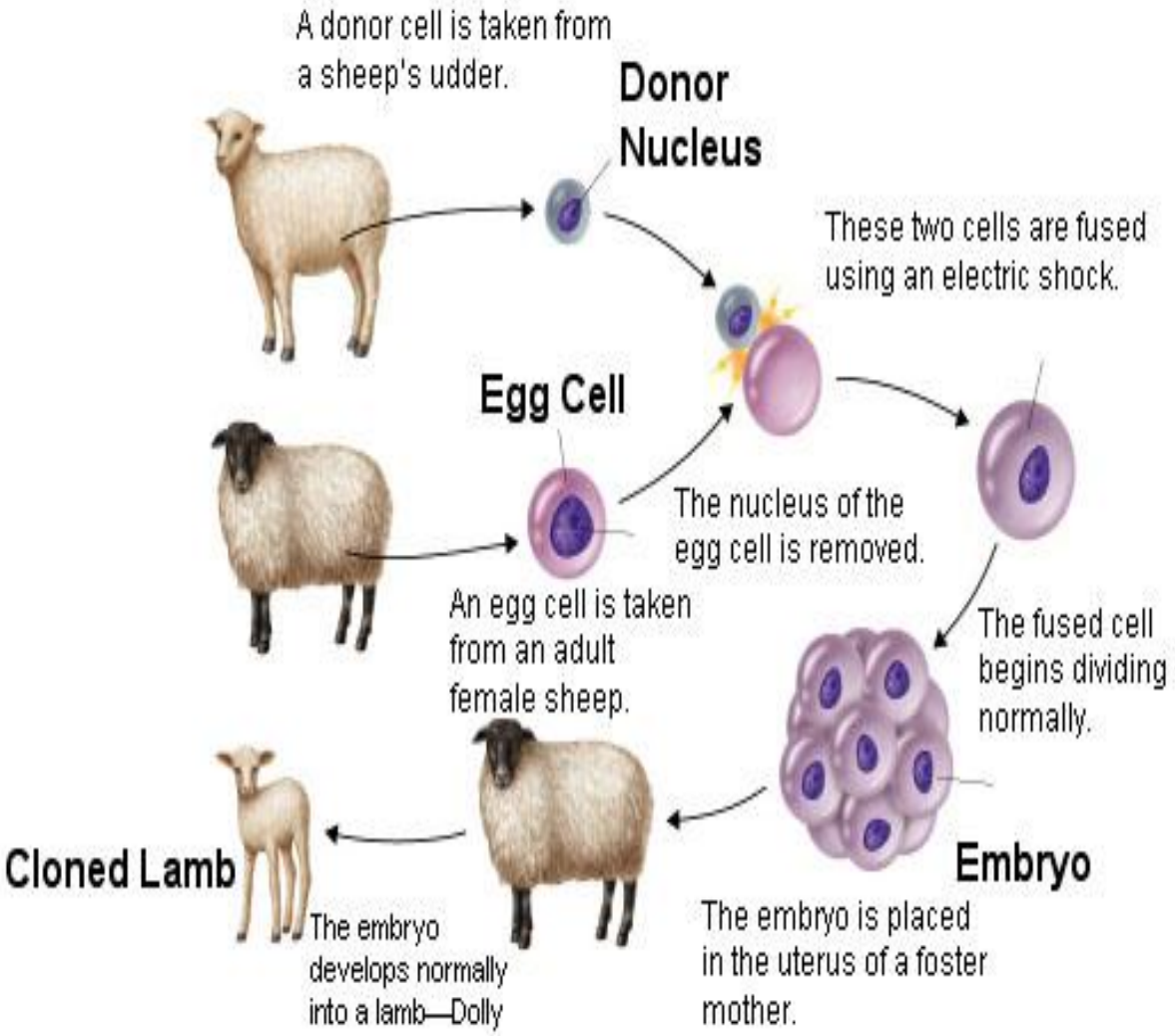
Scientists studying a particular gene often use bacterial plasmids to generate multiple copies of the same gene. Plasmids are self-replicating extra-chromosomal circular DNA molecules, distinct from the normal bacterial genome (see image to the right). Plasmids and other types of cloning vectors were used by Human Genome Project researchers to copy genes and other pieces of chromosomes to generate enough identical material for further study.

To "clone a gene," a DNA fragment containing the gene of interest is isolated from chromosomal DNA using restriction enzymes and then united with a plasmid that has been cut with the same restriction enzymes. When the fragment of chromosomal DNA is joined with its cloning vector in the lab, it is called a "recombinant DNA molecule." Following introduction into suitable host cells, the recombinant DNA can then be reproduced along with the host cell DNA.

Plasmids can carry up to 20,000 bp of foreign DNA. Besides bacterial plasmids, some other cloning vectors include viruses, bacteria artificial chromosomes (BACs), and yeast artificial chromosomes (YACs). Cosmids are artificially constructed cloning vectors that carry up to 45 kb of foreign DNA and can be packaged in lambda phage particles for infection into *E. coli* cells. BACs utilize the naturally occurring F-factor plasmid found in *E. coli* to carry 100- to 300-kb DNA inserts. A YAC is a functional chromosome derived from yeast that can carry up to 1 MB of foreign DNA. Bacteria are most often used as the host cells for recombinant DNA molecules, but yeast and mammalian cells also are used.

Reproductive Cloning

Reproductive cloning is a technology used to generate an animal that has the same nuclear DNA as another currently or previously existing animal. Dolly was created by reproductive cloning technology. In a process called "somatic cell nuclear transfer" (SCNT), (Somatic **cell nuclear transfer** (**SCNT**) is a laboratory technique for creating a clonal embryo, using an ovum with a donor nucleus) scientists transfer genetic material from the nucleus of a donor adult cell to an egg whose nucleus, and thus its genetic material, has been removed. The reconstructed egg containing the DNA from a donor cell must be treated with chemicals or electric current in order to stimulate cell division. Once the cloned embryo reaches a suitable stage, it is transferred to the uterus of a female host where it continues to develop until birth.(Dolly (5 July 1996 – 14 February 2003) was a female domestic sheep, and the first mammal to be cloned from an adult somatic cell, using the process of nuclear transfer. She was cloned by Ian Wilmut, Keith Campbell and colleagues at the Roslin Institute near Edinburgh in Scotland. She was born on 5 July 1996 and she lived until the age of six. The cell used as the donor for the cloning of Dolly was taken from a mammary gland, and the production of a healthy clone therefore proved that a cell taken from a specific part of the body could recreate a whole individual. As Dolly was cloned from part of a mammary gland, she was named after the famously curvaceous country western singer Dolly Parton.



a third
uclear
ocyte
(developing egg cell) that has had its nucleus removed. The hybrid cell is then stimulated to divide by an electric shock, and when it develops into a blastocyst it is implanted in a surrogate mother. Dolly was the first clone produced from a cell taken from an adult mammal. The production of Dolly showed that genes in the nucleus of such a mature differentiated somatic cell are still capable of reverting back to an embryonic totipotent state, creating a cell that can then go on to develop into any part of an animal.)

Therapeutic Cloning

Therapeutic cloning, also called "embryo cloning," is the production of human embryos for use in research. The goal of this process is not to create cloned human beings, but rather to harvest

stem cells that can be used to study human development and to treat disease. This starts with the same procedure as is used in adult DNA cloning. The resultant embryo would be allowed to grow for perhaps 14 days. It's stem cells would then be extracted and encouraged to grow into a piece of human tissue or a complete human organ for transplant. The end result would not be a human being; it would be a replacement organ, or piece of nerve tissue, or quantity of skin. Stem cells (**Stem cells** are cells found in all multi cellular organisms. They are characterized by the ability to renew themselves through mitotic cell division and differentiate into a diverse range of specialized cell types. The two broad types of mammalian stem cells are: embryonic stem cells that are isolated from the inner cell mass of blastocysts, and adult stem cells that are found in adult tissues) are important to biomedical researchers because they can be used to generate virtually any type of specialized cell in the human body. Stem cells are extracted from the egg after it has divided for 5 days. The egg at this stage of development is called a blastocyst. The extraction process destroys the embryo, which raises a variety of ethical concerns. Many researchers hope that one day stem cells can be used to serve as replacement cells to treat heart disease, Alzheimer's, cancer, and other diseases.

In November 2001, scientists from Advanced Cell Technologies (ACT), a biotechnology company in Massachusetts, announced that they had cloned the first human embryos for the purpose of advancing therapeutic research. To do this, they collected eggs from women's ovaries and then removed the genetic material from these eggs with a needle less than 2/10,000th of an inch wide. A skin cell was inserted inside the enucleated egg to serve as a new nucleus. The egg began to divide after it was stimulated with a chemical called ionomycin. The results were limited in success. Although this process was carried out with eight eggs, only three began dividing, and only one was able to divide into six cells before stopping.

How can cloning technologies be used?

If the low success rates can be improved (Dolly was only one success out of 276 tries), reproductive cloning can be used to develop efficient ways to reliably reproduce animals with special qualities. For example, drug-producing animals or animals that have been genetically altered to serve as models for studying human disease could be mass produced.

Reproductive cloning also could be used to repopulate endangered animals or animals that are difficult to breed. In 2001, the first clone of an endangered wild animal was born, a wild ox called a gaur. The young gaur died from an infection about 48 hours after its birth. In 2001, scientists in Italy reported the successful cloning of a healthy baby mouflon, an endangered wild sheep. The cloned mouflon is living at a wildlife center in Sardinia. Other endangered species that are potential candidates for cloning include the African bongo antelope, the Sumatran tiger, and the giant panda. Cloning extinct animals presents a much greater challenge to scientists because the egg and the surrogate needed to create the cloned embryo would be of a species different from the clone.

Therapeutic cloning technology may some day be used in humans to produce whole organs from single cells or to produce healthy cells that can replace damaged cells in degenerative diseases such as Alzheimer's or Parkinson's. Much work still needs to be done before therapeutic cloning can become a realistic option for the treatment of disorders.

What are the risks of cloning?

Reproductive cloning is expensive and highly inefficient. More than 90% of cloning attempts fail to produce viable offspring. More than 100 nuclear transfer procedures could be required to produce one viable clone. In addition to low success rates, cloned animals tend to have more compromised immune function and higher rates of infection, tumor growth, and other disorders. Japanese studies have shown that cloned mice live in poor health and die early. About a third of the cloned calves born alive have died young, and many of them were abnormally large. Many cloned animals have not lived long enough to generate good data about how clones age. Appearing healthy at a young age unfortunately is not a good indicator of long-term survival. Clones have been known to die mysteriously. For example, Australia's first cloned sheep appeared healthy and energetic on the day she died, and the results from her autopsy failed to determine a cause of death.

In 2002, researchers at the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts, reported that the genomes of cloned mice are compromised. In analyzing more than 10,000 liver and placenta cells of cloned mice, they discovered that about 4% of genes function abnormally. The abnormalities do not arise from mutations in the genes but from changes in the normal activation or expression of certain genes.

Problems also may result from programming errors in the genetic material from a donor cell. When an embryo is created from the union of a sperm and an egg, the embryo receives copies of most genes from both parents. A process called "imprinting" chemically marks the DNA from the mother and father so that only one copy of a gene (either the maternal or paternal gene) is turned on. Defects in the genetic imprint of DNA from a single donor cell may lead to some of the developmental abnormalities of cloned embryos.

TRANSGENIC ANIMAL

Transgenic animal are defined as animals which have incorporated foreign DNA into their germ line and are thus able to transmit this DNA to their progeny. At the moment direct microinjection of DNA into the pronuclear of fertilized one cell stage embryos is the method of choice for the generation of transgenic livestock.

Transgenic animals may also be defined as the animals that contains the foreign or introduced genes are called transgenic animals. The foreign or introduced genes are referred to as transgenes to distinguish them from endogenous genes.

A program for the production of transgenic animals can be subdivided into five main phases:

- Preparation of donor animals, isolation of fertilized oocytes and visualization of pronuclei.
- Preparation of DNA solution for injection.
- Microinjection of DNA solution into pronuclear.
- Transfer of injected zygotes into synchronized recipients.
- Screening of new born animals for integration of the transferred gene.

Dolly is a great example of first cloning sheep in the world

Dolly a cloned sheep, is a wonderful, fantastic, glorious and spectacular creature in the field of biotechnological by the world scientists. It has created a tremendous breakthrough in the dream land Biotechnological Avenue. It brings an immense impetus in the thought of human and as well as, conventional approaches prevailing in the planet. It has come into reality after a serious research and devotion for long 10 years at the Roslin institute, Edinburgh by Dr. Lan Wilmut and his co-workers in the year 1997. They have attempted 272 times before they have succeeded to produce the cloned sheep-dolly.

Major genes commonly found

- The halothane gene in pigs
- The double number gene in beef cattle
- Dwarf genes in broilers
- The Booroola gene in sheep

Transgenic mammal's production

A restrictive definition of transgenic animal relates to those animal that have integrated foreign DNA into their germ line as a consequence of experimental introduction of DNA, usually by microinjecting recombinant DNA directly into pronuclei of fertilized eggs.

Transgenic mammals are produced by microinjecting recombinant DNA directly into the pronuclei of zygotes shortly after fertilization using a concept and methodology developed earlier. This method allows virtually any DNA molecule to be introduced into the chromosomal complement of the zygote and therefore, into each of the cells of the resulting animals.

The ability to introduce genes into the germ line of mammals is one of the greatest technical advances in recent biology. The results of gene manipulation are inherited by the offspring of these animals. All cells of these offspring inherit the introduce gene as part of their genetic make-up. Such animals are said to be transgenic. Transgenic mammals have provided a means for studying gene regulation during embryogenesis and for studying the intricate interactions of cell in the immune system. The whole animal is the ultimate assay system for manipulated genes which is direct complex biological processes. In addition, transgenic animals provide exciting possibilities for expressing useful recombinant proteins and for generating precise animal models of human genetic disorders.

Transgenic mice are now routinely produced and studied in laboratories throughout the world. Over 50 different genes have already been transferred into recipient mouse eggs or embryos and stably maintained in adult mice and their progeny.

One of the most encouraging results of these studies on transgenic mice is that the transgenes have usually exhibited their normal patterns of expression and regulation. Transgenic sheep pigs, rabbits and chicken have also been produced.

Two methods are predominantly used to produce transgenic animals:

1. Microinjection of DNA into the pronuclei of fertilized eggs and
2. Injection of pre-implantation embryos with retroviral vectors

Characteristics of X- and Y-chromosome bearing spermatozoa

Some different between X and Y sperm

Parameter	Difference
DNA	Less in Y sperm
Size	X sperm is larger than Y
Identify	Y chromosome fluoresces
Motility	Y sperm is faster than X
Surface charge	X sperm migrate to cathode

There are two other laboratory methods used for the separation of animal and human X and Y sperm which are reproducible and clinically applicable:

- Albumin separation- this yield 75-80% Y sperm
- Sephadex filtration- this yields 70-75% X sperm

The separation of X and Y sperm is mainly based on the following

- Differences in the weight, density or size of the X and Y chromosomes as a result of differences in the size of different components of the sperms.
- Differences in the haploid expression of X and Y chromosomes as a result of differences in the nature of sperm components.