GASTRULATION IN FROG

The process of transformation of blastula into gastrula is called Gastrulation.

During gastrulation single layered blastula converts into three layered blastula by a series of dynamic cellular movements MORPHOGENETIC MOVEMENTS

The dynamic cellular movements involving the reorganization and rearrangement of cells (blastomeres) during gastrulation are called morphogenetic movements. These movements leads to the formation of future or presumptive germ layers like ectoderm, mesoderm, endoderm

Types of morphogenetic movements occurs during Gastrulation

1. **Epiboly**:- it is a type of morphogenetic movement where the cells of epidermal ectoderm(micromeres) multiply and spread all over the embryo and covering the macromeres, except at the blatopore. Due to the epiboly some of the yolk filled macromeres projecting out of the blastopore temporarily, this is called yolk plug and stage is called yolk plug stage.

Or

It is also defined as the process of overgrowth of micromeres on the macromeres.

2. **Emboly:**- it is a type of marphogenetic movement occur during gastrulation where cells(blastomerers) move into the interior from the surface.

Emboly represents inward movement of blastomeres or ingrowth

Invagination – It is defined as the active infolding or inpushing of blastomeres (macromeres) on one side of the blastula.

In frog, invagination takes place just below the grey cresent invagination results in the development of a new cavity called archenteron or gastrocoel. The invaginated cells occupy the floor and lateral side of archenteron, which forms endodermis.

The gastrocoel opens outside through the opening called blastopore. The margins of the blastopore are called lips, the dorsal margin is called dorsal lip, ventral margin is called ventral lip and lateral margin is called lateral lip

• **Convergence:**- it is defined as the directional movement of micromeres towards a particular point that is towards the dorsal lip of the blastopore.

During convergence micromeres at the animal pole proliferated and migrate towards the dorsal lip.

- Involution ("rolling in" movements):- It is inward rolling
 or rotation of the micromeres near the dorsal lip of
 blastomere into the gastrocoel. The involuted
 micromere occupy the roof of the gatrocoel and forms
 chorda mesoderm. The involution of micromeres
 enlarges the size of the gastrocoel . as the gastrocoel
 enlarges, the size of the blastocoels reduces, finally the
 blastocoels disappear.
- **Divergence**: it is the spreading movement of involuted blastomeres(micromeres) from the point of involution(i.e tip of the blastopore) with in the wall of the archenteron.
- Extension: It is the stretching of the converging notochordal and mesodermal cells in the gastrula.
 Due to epiboly, some of the yolk filled macromeres projecting out of the blastopore temporarily. This is called yolk plug and the stage is called yolk plug stage.

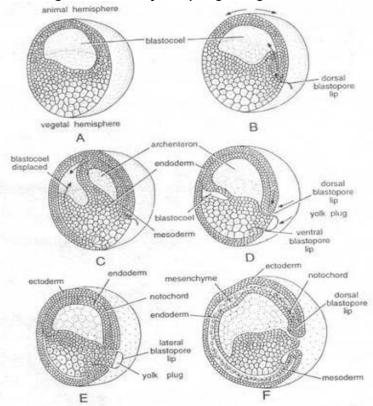


Fig. 15. Gastrulation in frog. A-Late Blastula, B-Beginning of gastrulation, C & D-middle garrula stages, E & R-Late gastrula stages,

Gastrulation Process in Frog

STRUCTURE OF GASTRULA

Gastrula is a three layered embryo consists of 3 germ layeres namely Ectoderm, Mesoderm and Endoderm

A mature gastrula is oval in shape.

The cavity of gastrula is called Archenteron.

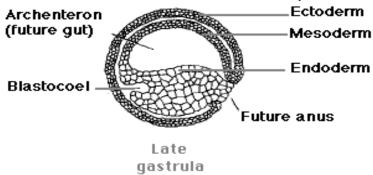
The archenteron opens exterior by blastopore, the blastopore has dorsal, ventral and lateral lip.

The blastopore is plugged by the yolk plug. It is composed of yolky macromeres.

The surface of gastrula is composed of micromeres this forms the ectoderm. The mid dorsal line of micromeres forms the neurectoderm and the remaining ectoderm forms epidermal ectoderm.

The roof of the archenteron is composed of micromeres which forms the chorda mesoderm. The floor of the archenteron composed of yolky macromeres which forms ectoderm.

In between the ectoderm and endoderm there is a layer of mesoderm is present. It is formed by the cells which involuted at the ventral and lateral lip of the blastopore.



GATRULATION IN CHICK

Gatrulation in chick is highly prolonged and modified due to the presence of large amount of yolk. Gastrular movement (morphogenetic movement) are responsible for formation of embryonic membranes

Formation of endoderm or Hypoblast

Presumptive endoderm or hypoblast is formed during gastrulation by the delamination of large yolk cells from the

inner surface of epiblast. These form a distinct layer in the subgerminal cavity above the yolk

Formation of Mesoderm

The cells of notochord and mesoderm migrate inward and form a layer in between the epiblast and hypoblast. These movements are leads in the form of primitive streak. Thus the three embryonic layers are established the uppermost or epiblast now forms the ectoderm, the median layer mesoderm and the lower one endoderm.

• Formation of primitive streak

The endoderm is migrated to the definite position, the central area of blastoderm is left with prospective somatic mesoderm, prechordal mesoderm, neurectoderm and the epidermal ectoderm.

o Formation of initial primitive streak

Later mesoderm begins to converge towards the middle line from a point about a quarter of the way from the anterior end of area pellucida (epiblast) towards the growth centre in the posterior quadrant to initiate in the formation of a thickening called the primitive streak

By the twelth hour the intermediate streak is formed is about one half the length of the circulared area pellucida, it continoues to grow in length, elongated backward and its posterior end. The oval area pellucid becomes pear shaped to accommodate the lengthening streak.

o Formation of definite primitive streak

By the 19th hour of incubation there is forward stretching of primitive streak.

This primitive streak contains the presumptive lateral plate mesoderm, prechordal mesoderm and notochordal materials. The movements in the blastoderm leading to the formation of primitive streak may be called pregastrulation movements.

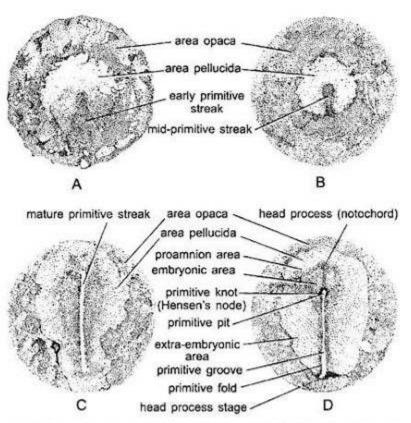


Fig. 38.5. Surface view of chick blastoderm showing development of primitive streak (gastrulation) and head process (neurulation). A-Initial streak (stage 2); B-Intermediate streak (stage 3); C-Definitive streak (stage 4); D-Head process stage (stage 5 embryo of 19 to 22 hours of incubation).

NEURULATION IN FROG EMBRYO

The formation of neural tube (neutralization) and its further differentiated into brain, spinal cord and different sensory organs such as olfactory (nose), optic(eye) and auditory(ear) organs, are collectively included in the process of neurogenesis.

The process of neuralation, specifically is the process of neural tube formation. In frog it includes following processes all of which occur in a simultaneous manner

Neural tube formation

At the end of gastrulation, when the yolk plug finally disappear and the blastopore closes to a dorso ventral slit soon

- afterward the presumptive area of the nervous system become differentiated from the rest of ectoderm in the form of pear shaped medullary or neural plate
- The ectodermal epithelium moves towards the dorsal side of the embryo, at the same time the cells of neural plate changes its shape become elongated and arranged them selves into a columnar epithelium
- During this process the embryo lengthens along its anteroposterior axis, at the same time the edges of the neural plate become thickened and raised above the general level as ridges called neural fold.
- The neural folds become higher, so that the neural area contained between them forms a wide neural groove. Subsequently, the neural folds meet each other in dorsal middle line and fuse, in this way the neural tube is formed.
- Finally some regionalization becomes visible in the neural tube with sub division of the brain into prosencephalon (fore brain) mesencephalon (mid brain) rhombencephalon(hind brain).

Tabulation of chorda- mesoderm

At the time of the closure of the blastopore the chorda – mesodermal mantle seperates itself from the endoderm and the mesoderm attains its definite position between the endoderm and ectoderm.

Formation of Notochord

- After the speration prechordal plate from endoderm a narrow rod of cells, the remainings of notochord seperates from the rest of the chorda-mesodermal mantle.
- Shortly after fluid- containing vacuoles appears in the notochordal cells.

Differentiation of mesoderm

Simultaneously the tip of mesoderm at each side of the notochord thickens and subdivides in transverse plane beginning at the end into a series of cell masses or somites.

> Tubulation of Endoderm

As neurulation begins the free margin of the endoderm unite in the dorsal middle line beneath the notochord to complete the formation of the definitive gut (enteros). The floor of the enteron has thick yolk filled cells. Later the lungs, liver and pancreas develop from evaginations from the gut.

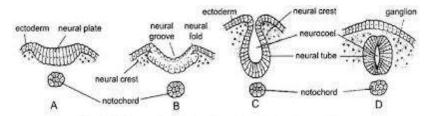


Fig. 37.7. Stages in the formation of neural tube in amphibians.

Unit-8 Dairy

A dairy is a business enterprise established for the harvesting or processing of animal milk mostly from cows or goats, but also from buffaloes, sheep, horses or camels for human consumption. With industrialization and urbanization the supply of milk become a commercial industry with specialized breeds of cattle being developed for dairy as distinct from beef or draught animals.

Most countries produce their own milk products, the structure of the dairy industry where is in different parts of the world in major milk producing countries most milk is distributed through whole sale markets.

Composition of milk and nutritive value of milk

Milk is produced by mammary gland. Its secretion is stimulated by prolactin in the mother at the time of birth of a calf (young one) adrenal hormone is also essential for lactation.

Milk is an emulsion of fat in a continuous phase. The dispersed phase consists of fat globule of varied diameter. Fat content varies from 0.1 to 10%.

The fat globules are surrounded by layers of protein phospholipids carotenes and cholesterol. This layer prevents the globules from coalescing together into large globules, protective layer may be broken by churning when the fat globules coalesce to form butter.

Milk /fat/ butter consists of glycerides of butyric caproic acid and capric acids. Which posses characteristic odour. The continuous aqueous phase consists of Carbohydrates(lactose) and a colloidal suspension of casein, stabilized by lactalbumin and lactoglobulin.

Lactose form the largest constituent of milk next only two water, fat soluble vitamins and water soluble minerals are present along the milk.

Amylase, catalase, peroxidase, lipase, phosphotase, galactase, lactase and aldehydase are important for the enzymes. These enzymes are destroyed due to pasteurization.

The white appearance of milk in reflected light and its opacity in transmitted light is due to emulsified fat and the colloidal calcium phosphate and caseinate.

Creamy colour is due to the presence of Keratin in the dispersed phase and of riboflavin in aqueous phase.

The flavour of milk is pleasant and sweet. Milk containing 3.5% fat and rich in lactose has a butter flavour.

constituent	Sindhi	Buffalo
Water	86.07	83.63
Fat	4.90	6.55
Protein	3.42	3.38
Lactose	4.91	5.23
Minerals	0.70	1.21

Nutritional value of milk

Milk is a complete food for infants up to 6 months of age. After which acts as a supplement to other food. Milk is a good source of phosphorus calcium and vitamins.

Cow milk is easily digestible, 50% of its caloric value is contributed by fat, 20% by lactose and 21% by protein. Milk is rich in vitamin A, D, B1 and B2.

Milk is rich in enzymes like amylase, catalase, peroxidase, lipase, phosphotase, galactase, lactase and aldehydase.

Proteins like lactalbumin, lactoglobulin which are also antibodies are Richey present. The additives like phospholipids, carotene, cholesterol are present.

Milk sugar like lactose a disaccharide constituents about 4 to 7% of milk, its hydrolysis gives glucose and galactose.

Milk consists of fat of about 4 to 7% called butter fat composed of triglycerides. Milk protein like cascin, globulins, immunoglobulins, proteases and peptones.

salts like, calcium chloride, citrate, sulphate and bicarbonate of sodium and potassium are present there are also traces of iron and copper.

Cattle breeds

A breed is group of one species of animals, which have the same descent and are similar in body shape, size and structure.

Examples: Amrithmahal, Kangayan, Malvi, Siri, Hallikar, Khillari etc..

Indigenous Draught breeds of cattle Draft breed:

Hallikar:- They are breeds mainly meant for transport and field work.

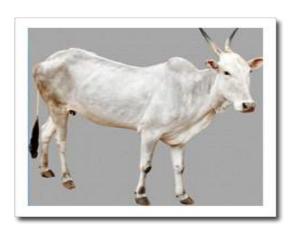
- Originated from the former princely state of
- Vijayanagaram, presently part of Karnataka.
- The colour is grey or dark grey.
- Compact, muscular and medium size animal with prominent forehead, long horns and strong legs.
- The breed is best known for its draught capacity and especially for its trotting ability.
- Both bulls and cows have horns.
- The cows are average milk producers and an average can produce about 542kg of milk/lactation.
- Green fodder, chiefly comprises finger millet, grass sorghum or pearl millet.

uses

- The bulls are known for their strength and endurance and are primarily used for draft purposes.
- Along with normal draught purpose, the Breed is also used for cart racing.
- It is considered as one of the premier draft cattle breeds in India. That cows are always milkers.

Red Sindhi (Milch breed)

Examples: Gir, Red sindhi, Sahival and





Deoni.

They are breeds mainly meant for milk production.

- This breed is otherwise called as Red Karachi and Sindhi and Mahi.
- Originated in Hyderabad and Karachi (Pakistan) regions of undivided India and also reared in certain organized farms in our country.
- Colour is red with shades varying from dark red to light, strips of white.
- Milk yield ranges from 1250 to 1800 kgs per lactation.
- Bullocks despite lethargic and slow can be used for road and field work.
- They having short, curved, lyre shaped horns.
- The bulls are usually of a darker colour than the cows.
- The heritability for milk yield is 0.30 to 0.35.
- Age of first calving is 39 to 50 months.
 Calving interval is 425 to 540 days.

Uses

- It has been used to improve beef and dual purpose cattle in many tropical countries.
- High milk production helps give a fast growing cough which is ready for market at 1year.
- Cross breeding method is commonly used for improving cattle breeds. It is used for producing more yield of milk



Ongole (dual purpose breeds)

Examples: Hariana, ongole,etc..

They are used for both work and production of milk.

- Otherwise known as Nellore.
- Home tract is Ongoletaluk in Guntur district of Andhra Pradesh.
- Large muscular breed with a well developed hump.
- Suitable for heavy draught work.
- White or light grey in colour.
- Average milk yield is 1000 kgs per lactation.
- Animals of the Ongole breed were extensively exported to USA for beef production and also Brazil for beef and milk, Sri Lanka for drought etc..
- The Breed produces moderate milk with an average of 798 kg per lactation.
- In average fat percentage is about 3.79 percent.

uses

- The bulls commonly used in bull fight due to their strength and aggressiveness in Mexico.
- Tthey are also used for traditional fight in Andhra Pradesh and tamilnadu.
- Angole cattle were the first Indian breed of cattle to gain worldwide recognition.

Ongole cows stay close to their calves to protect them from Predatory animals.

Exotic dairy breed:

They are breeds originated outside india and then imported to our country.

Examples: Holstein, Ayrshire and Redden etc..

Holstein Friesian

- Originated from the northern parts of Netherlands, especially in the province of Friesland.
- Largest dairy breed and ruggedly built is shape and possess large udder.
- Breeds have typical marking of black and white that make them easily distinguishable.
- The average milk production of cow is 6000 to 7000 kg per lactation.
- The adult males and females about 800 to 900 kg and 500 26 50 kg respectively.
- The healthy calf weighs 40 to 50 kg or more at birth.
- Generally breeders plan for HF to calve for the first time between 21 and 24 months of age.

The gestation period is about 9 and half month.

Breeders important specialised Dairy holstein from the United States to cross with the European black and whites.

Uses

It is an adult purpose breed, used for both dairy and beef.

The exotic breed of cattle are high milk production.

Red Dane (Danish red)

Orginated in Denmark.

- Body colour of this Danish breed is red, reddish brown or even dark brown.
- It is also a heavy breed.
- The lactation yield of Red Dane cattle varies from 3000 to 4000 kg.
- They having great heat tolerance.
- It having fat content is about 4% and above.
- The calving interval 13 months.
- Both bulls and cows usually may have small horns.
- This breed is disease resistant and we'll adopted to varying climates.
- Mature males weighing up to 950kg and mature female is about 600kg. Uses

The Danish red cattle are a dual purpose animal.

They are raised for both milk and meat production.



Buffalo breeds

Examples: Murray, Jaffrabadi, Bhadawari, Niki Ravi, surti, Nagpur.

Surti: Also known as Deccani, Gujarati, Talabda, Charator and Nadiadi.

- The breeding tract of this breed is Kaira and Baroda district of Gujarat.
- · Coat colour varies from rusty brown to silver-grey.
- · Tail is fairly long and back is straight.
- The horns are sickle shaped, moderately long and flat.
- The peculiarity of the breed is two white collars, one round the jaw and the other at the brisket region
- The milk yield ranges from 1000 to 1300 kgs per lactation.

The peculiarity of this breed is very high fat percentage in milk (8-12%)

Uses

They are mainly used for milk production purpose.

The bullocks are used for good light work.



Nagpuri

- This breed is also called as Elitchpuri or Barari.
- The breeding tract of this breed is Nagpur, Akola and Amarawati districts of Maharashtra.
- These are black coloured animal with white patches on face, legs and tail.
- The horns are long, flat and curved, bending backward on each side of the back. (Swaord shaped horns).
- The bullocks can be used for heavy work.
- The milk yield ranges from 700 to 1200 kgs per lactation.
- The age at first calving is 45 to 50 months with an intercalving period of 450 to 550 days.
- It is an central Indian breed.
- They having 7.7% of fat soluble in milk.
- These breeds are slow movement and less active.
 Uses

It is very good for milk production.

Male animals are used for draft purposes but it works slow than bull.

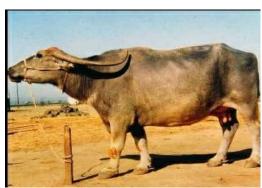
Diseases in Cattle

Mastitis is the inflammation of the memory gland and udder tissue and is a major endemic disease of dairy cattle.

It is most often transmitted by contact with the milking machine and through contaminated hands or other materials.

causes

It is a multifactorial disease, since the infectious infection depends on germs, environmental conditions and the characteristics of the cow. The microorganisms invade the tissue breast causing on inflammation of the gland.they are caused by two types of mastitis,



1) contagious mastitis:

This is caused by microorganisms Streptococcus agalactiae and staphylococcus aureus. They are transmitted during milking through contaminated milking machinery. the caof or the improper handling by the workers it caused a reduced to level of milk.

2) environmental mastitis:

This is caused by microorganisms environmental streptococci and coliforms. They are transmitted between period of milling and dry periods when the gland does not produce milk.

Symptoms:

This is an inflammation of the affected udders the animals feel pain when touched.

The milk is altered and blood is sometimes seen as well as flakes, clots and colourless puas.

Fever, lower milk production and loss of appetite.

The microbial count and somatic cell is high.

Diagnosis:

The milk sample are collected and conducted the following tests,

Somatic cell count

Bacterial culture of milk and other test.

Treatment:

Treatment depends on the cause of the microorganisms and whether it is subclinical are clinical this can be done using intramammary antimicrobials.

Prevention:

Teat disinfection before and after milking

good hygiene during milk in period

good milking machine for also used

call out a veterinarian to take information

given a good nutrition, water and quality bedding to the cattles

good ventilation clean and dry tests

keeping cows standing after milking.

Anthrax:

Anthrax is an acute disease having rapidly fatal course. it is the oldest disease known in the cattle disease. It is characterized by septicemia and sudden death.

Causes

The causative agent of this disease is bacillus anthracis.

the organisms is a relatively large, rod-shaped and non motile.

the animals get infection by ingestion of food and directly from animal to animal.

Symptoms

Shivering fits with rise of temperature the temperature of animal body go up to 106 degree fahrenheit.

Rumination stops, eye become red, extremities get cold

breathing is difficult

abdominal pain and tympanites

dung is stained with blood and rectum protrudes

bloody discharge from mouth nostrils and rectum. the discharge is tarry in colour

the animal dies within 24 hours, if the disease is in acute form.

Treatment

The treatment is usually not possible in acute cases but subacute cases are treated with antibiotics and antianthrax serum.

Penillin and streptomycin in large doses are recommended

Annual vaccination of the animals recommended in the endemic areas.

Prevention:

Good hygiene is the most important single factor in the prevention of spread of Anthrax

destruction of contaminated material and disinfection of equipments and animal shed are is also necessary vaccination in endemic areas is very important to control the diseases

as the population of disease causing organisms takes place in the presence of oxygen, the vegetative forms present in the tissues and body Fluids will die if the caracass is not open but is burnt or buried deep with lime.

Septicemia:

It is also called hemorrhagic septicemia.

this is mainly occurs in cattle and buffaloes and also goats, camels, Horses and donkeys it is a severe bacterial diseases.

Causes:

The causative agent of this disease is starting stains of pasteurella multicoda

the diseases is spread through contact with infected animals, contaminated clothing, equipments and through injection or inhalation of bacteria.

The animals under stress or with poor body condition are believed to be more susceptible to p.multicoda infection.

Symptoms:

The infected diseases swollen neck and lower jaw

leathArgy, reluctant to move

nasal discharge

painful or difficulty breathing

excesive salivation, fever and finally become death.

Treatment:

The antibiotics is only effective when administered early

several of the sulfonamides and antibiotics such as penicillin and the tetracyclines can be used successfully in the early stages.

animals with elevated temperatures are isolated and treated intravenously with a soluble sulfonamide.

Diagnosis:

Diagnosis on the basis of blood smear and clinical findings.

Prevention:

Maintaining hurts in good physical condition and bearing the entry of animals

vaccination for haemorrhagic septicaemia is available

when favourable conditions for outbreaks are known to recur periodically.

In endemic areas the only practical ways to protect animals are by an organised program of vaccination.

Foot and mouth diseases:

It is Viral disease and it is highly communicable disease affecting cloven footed animals. it is characterized by fever, formation of blisters in tile mouth, udder, teats and on the skin between toes and above the hoofs.

in India the diseases widespread and assumes a position of importance in livestock industry.

causes

the disease is caused by a virus called foot and mouth disease virus.

it is spread by direct contact or indirectly through infected water, mannure, hay and pastures.

it is also conveyed by cattle attendants

the virus and gains entry into the bloodstream of animals through injury to the lining membranes of tongue intestine, clefts of hoofs and other similar parts.

Symptoms:

Rise of body temperature, dry muscle, dullness, depression, shivering, staring coat, loss of appetite and stoppage of rumination.

slight constipation

dribbling of saliva from tile mouth.

formation of blisters on the tongue and cheeks.

shaking and kicking of legs and lameless.

Vesicles at the cleft of the hoof become ulcer like and may get fly blown.

the milk is comes down in quantity and quality and the milk coagulates on boiling

the infected animals cannot be put to hard work especially in the sun and it gasps from breath a condition known as panting.

Treatment:

No therapeutic agents have been found till now to cure foot and mouth disease

a common and inexpensive dressing for lessons of feet is a mixture of Coal Tar and copper sulfate in the proportion of 5:1 some other measures to treat the disease are given below

clean the wounds and ulcers in the mouth, udder, teats and feet with 2% potassium permanganate lotion or alum water

decoction of babool bark for gargling the mouth and washing the ulcers may also be effective.

apply boric acid mixed with glycerine to ulcers in the mouth.

foot bath with a disinfectant solution such as cresol or phenol (1:100) may be used.

Sores on the udder and teats of milch cows should be kept clean and dressed with boric ointment.

Management of dairy form

The building in which dairy cattle are dept is called dairy house.

Selection of sight should consists of dry place, elevated place drainage facility and

good water supply.

The dairy house should contain cowshed, suckling calves room, feeding Room, milkroom, washroom, loafing room.

The cowsheds can be arranged in single room if the cows are less than 10 or in a double row if the cows are more than 10, in the middle there is a passage.

In the double row system the cows should be arranged face to face or tail to tail

MANAGEMENT OF COW

the cow has three stages in its Life. They are,

- 1.new born calf
- 2.heifer
- 3.milking cow

CARE OF NEW BORN CALF

As soon as the calf is delivered, the mucous is removed from the nose and mouth and it is cleaned.

If the calf does not start breathing artificial respiration should be given by pressing the chest .

Another method is to hold calf by the rear legs hand lift from the Flore with the head down, this may be respected several times.

The naval cord of the calf is tied about 2.5 cm away from the body and cut about 1 cm below the ligature. Appt tincture iodine and repeated it 2-3 days. This prevent infection.

Feed colostrum with in 15 minutes of calving.

Identification - metal ear tags or button with letters and numbers may be inserted in the year.

Dehorning- by use of hot iron to removal of horns with in the age 3 to 10 days.

* Note- colostrum = it contains low fat, high protein, vitamin s and minerals .this helps to protect the calf against various diseases as it contains antibodie.

MANAGEMENT OF HEIFER

Heifer is the stage of the cow which does not have yielded calf. It has got an age 15 to 18 months from the date of calving.

Usually heifers at kept in indoors up to 9 to 12 months, then they are raised outdoors and protection from adverse climatic conditions, rains, sun etc, are essential.

The heifer cow is still growing and as not reached her adult body weight. So, extra growth ration as to be provided to milking heifer cow, is through out first location.

Management of milk cow

1)Feeding management

Adequate quantity of roughages expecially greens are usually fed 4 to 5 times a day.

The concentrates her fed usually at the times of milling, this helps in, led down, of milk.

Concentrate feed should the given at the rate of 1.5 kg per animal as maintainance ration.

1 kg of concentrate feed should be given for every additional 2.5 kg of milk yield.

1.0 to 1.5 kg concentrate feed should given to the pregnant cows from 7th months of pregnancy.

A milking cow requires 15 to 25 kg of green fodder and 3 5 kg of paddy and straw per day in addition to the concentrate feed.

2)Breeding management

The heifer comes to heat at about 18 months of age.

A period in the oestrous cycle during which cow will mate is called the oestrum as heat period.

The oestrous cycle averages about 21 days in length.

The heat period will usually last from 14 to 18 Hours, but may vary and if ranges from 12 to 24 hours in length.

Dairy cows usually comes to heat about 30-40 days after calfving.

The gestation period of cow is about 280 daus.