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Calf Rearing

Bio-Pesticide

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Bio-Pesticide:
A Viable Option for Farmers

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Electronics, space travel and genetic manipulations have, no doubt, opened new vistas for mankind but at the same time we have been ignorant to some degree, about our diverse biological resource, which constitute the apogee of the evolutionary process. Intensive agriculture with the use of agro-chemicals in large quantities has no doubt resulted in many fold increase in productivity of farm commodities, but adverse effect of these chemicals are visible on soil structure, soil micro-flora, quality of water, food, fodder and food materials. Future agriculture warrants on eco-friendly or organic farming which should create an ecological balance and microenvironment suitable for health and growth of soil micro-flora, plants, animals, farm workers and ultimately the vast population which consume the farm produce. Now it is high time that we should seriously think about alternative pesticide and fungicide which are safe to both human and environment. Botanical or bio-pesticide is one such way in this direction which is effective, commercially viable and inexpensive.

The pesticidal value of few plants is briefly mentioned below:

A. Sweet flag (*Acorus calamus*): It is semi-aquatic rhizomatous perennial herb belonging to the family Araceae, and is cultivated for its rhizome. The dried rhizome is powered and used for destruction of bedbugs, moth, lice and insect pests.

B. Siris tree (*Albizia lebbeck*): This is a large, erect, unarmed spreading tree belonging to the family leguminoseae is common all over India. The seeds petioles, leaflets and pods are toxic to cotton stainer bug adults. Leaves contain caffeic acid, alkaloids, kaempferol and quercetin and bark contain tannins.

C. Garlic (*Allium sativum*): This herbaceous plant belongs to Liliaceae family is believed to be indigeneous to the mountain regions of Central Asia. Presently garlic is cultivated in the tropical and temperate regions of the world. The whole plant, bulbs, leaves and flowers are either used fresh or dried. The pesticidal activity of garlic is due to the presence of an acrid volatile oil which contains diallyl disulphide, diallyl trisulphide and sulphoxides derived from

allicin present in it. Garlic oil is hundred percent effective on several species of mosquitoes, houseflies and major insect pest. A mixture of garlic and neem oil suitable in the control of coconut mite (*Eriophis*).

D. Aloe (*Aloe succotrina*): A wild perennial herb belonging to family Liliaecae is of great medicinal value. The leaves contain barbolin, chrysophenol, glycoside, aglycone, aloe-emodin, etc. Aloe has antifungal, antibacterial properties when mixed with castor cake. Aloe in combination with other plant products also control a variety of pest efficiently.

E. Custard apple (*Annona squamosa*): The seeds of custard apple possess insecticidal and anti-feedant properties. The powdered seeds are used to destroy worms, seeds are reported as contact poison to flies, aphids and several beetles.

F. Indian neem tree (*Azadirachta indica*): The much talked about tree which was patented by an American company and released belong to Meliaceae family. The most biologically active constitutent of the neem is the highly oxygenated azadirachtin A. The azadirachtin present in fruit, kernel, oil cake and leaves act as repellent, growth retardant and opposition inhibitor when insects are treated with water extracts. Neem oil is used in soaps, tooth pastes (germ killer), hair tonic for killing lice (parasite killer). Margocides, Achook, Bioneem, Neeazal F, etc. are some of the commercial botanical pesticides available in the market.

G. Pyrethrum (*Chrysanthemum cinerariifolium* and *C. coccineum*): The flower is the source of pyrethrins I and II sesquiterpenes, flabonoids, caronoids and is the safest insecticide. It is specially recommended for the control of household insects like houseflies, mosquitoes, cockroaches and for the protection of food grains.

H. Cinchona (*Cinchona officinalis*): The important alkaloids present in bark are quinine, quinidine, cinchonine and cinchonidine which are useful for the treatment of malaria. Cinchona alkaloids are used as preservative for fur, feather, wool and textiles.

I .Datura (*Datura metel*): Datura is a herb and belongs to family Solanaceae. It contains many alkaloids such as hysoyne, hyoscyamine and atropine which have insecticidal property. The growing plant is said to protect neighboring plants from insects.

Figure 1 Sweet flag

Figure 2 Aloe

Figure 3 Garlic

Figure 4 Custard apple

Figure 5 Siris

Figure 6 Indian Neem

Figure 7 Pyrethrum

Figure 8 Custard apple

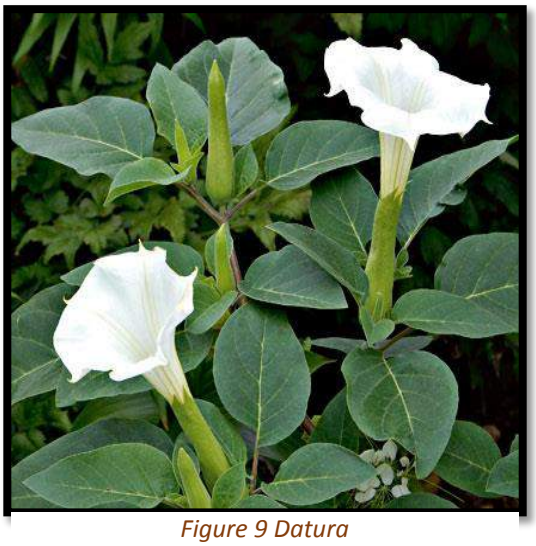

Figure 9 Datura

Figure 10 Mahua

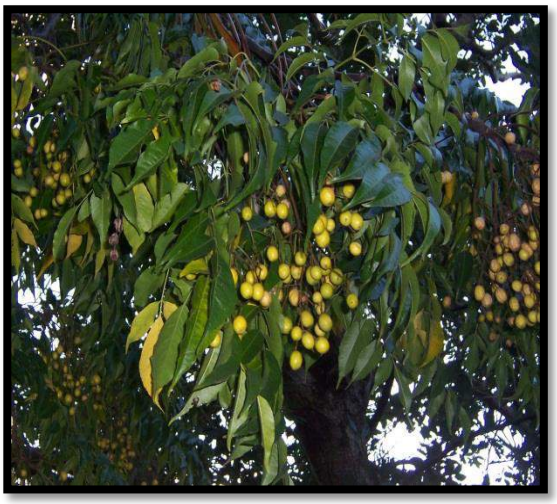

Figure 11 Maha Neem

J. Mahua (*Madhuca longifolia*): It is a large tree with dense spreading crown with dark grey or brownish leave and scaly dark belonging to family

Sapataceae. Seed contains saponines, leaves have beta carotene, sitoseterol and fruit contain nterpinodes. It has insecticidal and anti-bacterial properties and controls a wide variety of pests.

K. Maha neem tree (*Melia azadirachta*): It is a moderate deciduous tree found in sub Himalayan tracts, leaves of which protect books and woolen clothes. Leaf extracts are used against grasshoppers and locusts. Semi purified extract of this plant exhibited anti feeding, toxic, growth regulating, sterilizing and ovicidal effects on a number of insects. As in neem, melia is known to synthesize tetra and pentanotriterpinoids responsible for its bioactivity against insects.

L. Tobacco (*Nicotiana tabacum*): Tobacco is an erect glandular and pubescent herb belonging to the family Solanaceae, cultivated in India for its leaves. Tobacco extracts are generally used to eradicate lice, and ticks in animals. The active constituent in this plant is nicotine. Sulphate of nicotine is widely used as insecticide.

M. Tulsi (*Ocimum tenuiflorum*): It is a sacred herb for Hindus who consider it as an earthly manifestation of Goddess Vrindavani who is dear to Lord Vishnu. It contains citral, linalool, geranial, methyl chavinol, acimene, eugenol, etc. It has both repellent and herbicidal properties. The tenders leaves when eaten raw are preventatives of many fevers, sore throats, respiratory disorders, kidney stone, heart disorders, etc.

N. Karanja (*Pongamia pinnata* or *P. glabra*): Pongamia extract of leaves and kernel serve as natural pesticides. Chemical constituents of pongamia are ponaglabrone, dixetonepongamol, glabrin, karanijin, pongapin and kanjone.

O. Nirgundi (*Vitex negundo*): It is an aromatic large shrub or small tree belonging to family verbenaceae. Leava contain alkaloids namely nishindine and hrocotylene. Vitex leaf extract in combination with aloe, pongamia, castor and calotropis successfully control a variety of pests. Thus, these botanical pesticides act as direct toxicant, repellents, behavioral and physiological disruptant. The use of botanical pesticides will not only manage pests but also provide alternative and economically viable options to farmers at field level as compared to synthetic pesticides in long run of time.

Recent Trends In Feeding Management Of Dairy Calves

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Milk is the best naturally balanced food for the mankind. Many commercial entrepreneurs are facing many difficulties in procuring good pedigreed breeding stock in establishment stage of dairy farming. Instead of searching the good pedigreed replacement stock, raising their calf on scientific line of management. The newborn calf is not a ruminant. Pre ruminant refers to the period after birth when the calf is dependent on milk as its major food. At birth and during the first few weeks of life, the compartments of the digestive system (i.e. rumen, reticulum, and omasum) are undeveloped. In contrast to the mature cow, the abomasum of the newborn calf is the main compartment, constituting 60% of the total tissue weight of the stomach. Colustrum provides antibodies which are absorbed intact in the first few days of the calf's life. Bovine colostrum imparts passive immunity to newborn calves during the first 24 h of life and generally has been fed for the first 3 days after birth. Calf starter is gradually introduced at the rate of 50 to 100 g / animal / day and increase upto 500 g / animal / day. Present calves are the future cows. So, perfect time schedule in feeding of calf should be maintained to get optimum growth rate. Thereby, they will mature in time for breeding.

Introduction

Milk is the best naturally balanced food for the mankind especially in paediatric and geriatric age groups. A Very few numbers of dairy cows are reared by individual farmer in existing system of dairy farming. But the trend changes to commercial dairy farming. Commercial dairy farming involves scientific management principles and techniques of housing, breeding and feeding. Many commercial entrepreneurs are facing many difficulties in procuring good pedigreed breeding stock in establishment stage of dairy farming. Instead of searching the good pedigreed replacement stock, raising their own calf on scientific lines towards the breeding

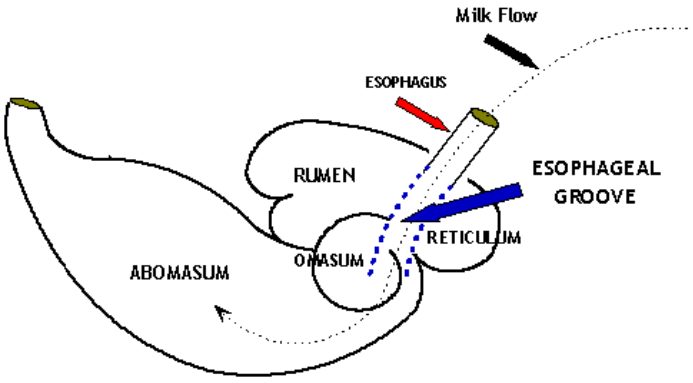
purpose is highly indispensible in current trend. So, success in Commercial dairy farming is highly dependent on the scientific feeding of calf.

Feeding of young calf

Reducing death losses of newborn calves to less than 5% and raising strong, healthy heifers large enough to breed at 14 to 16 months of age are sound management objectives. Calves stunted from underfeeding or diseases may not develop into healthy cows.

Significance of Oesophageal groove

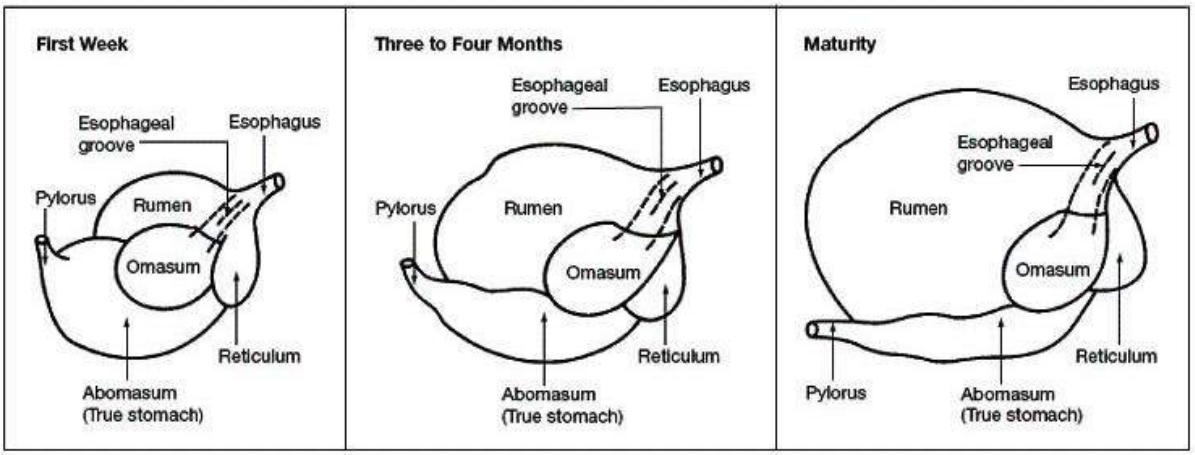
- The newborn calf is not a ruminant. Pre ruminant refers to the period after birth when the calf is dependent on milk as its major food. At birth and during the first few weeks of life, the compartments of the digestive system (i.e. rumen, reticulum, and omasum) are undeveloped.
- In contrast to the mature cow, the abomasum of the newborn calf is the main compartment, constituting 60% of the total tissue weight of the stomach. At this stage of life, the rumen is nonfunctional and the calf can not utilize some feeds digested by the adult. During nursing or feeding from a bucket, milk bypasses the rumen via the esophageal groove and passes directly to the abomasum.
- Reflex action closes the groove to form a tube-like structure, which prevents milk or milk replacer from entering the rumen. When milk is consumed very rapidly, some may overflow into the rumen.



Development of the Rumen

- The rumen remains undeveloped as long as the calf remains on milk, When calves begin consuming solid food, a microbial population becomes established in the rumen and reticulum. End products of microbial fermentation (i.e. volatile fatty acids) are responsible for the development of the rumen. This occurs as early as 3 weeks of age with most feeding programs.
- If grain feeding with or without forage is started during the first few weeks of life, the rumen will become larger and heavier with papillae

Development of bovine stomach compartments from birth to maturity



Feeding colustrum

- Colustrum provides antibodies which are absorbed intact in the first few days of the calf's life. Bovine colostrum imparts passive immunity to newborn calves during the first 24 h of life and generally has been fed for the first 3 days after birth.
- The immunoglobulins are absorbed in the body by the process of pinocytosis. After this age, globulins carrying antibodies are broken down by proteolytic enzymes in the process of exogenous digestion.
- Immunoglobulin IgG concentration in colostrum from Holstein cows beginning their first, second, or third lactation was similar. However, older animals had more IgG in colostrum. Its rate of disappearance from colostrum was greater in younger animals. Dry matter, ash, total protein, and whey protein concentrations decreased from the first to the third milking (24 h) postpartum. Protein was the most variable constituent between cows at the same postpartum time (***Oyeniyi, O.O. and Hunter, A.G.1978***).
- The colustrum also contains anti tryptic enzyme which may help in the protection of whey protein from the proteolysis. It also has a laxative effect in removing muconium.
- The colustrum should be fed at the rate of one tenth of body weight of the calves. If mother's colustrum is not available, two eggs may be mixed with milk along with 30 ml castor oil and it can be given to the calves.
- In addition, it is necessary to inject the calf with dam's serum for augmenting the antibody titre in the body, particularly the buffalo calves.
- Surplus colostrum is unmarketable and available in quantities sufficient to feed heifer calves through 28 to 35 days of age. Colostrum can be preserved conveniently for future use by brief refrigeration, freezing, or storage at ambient temperatures

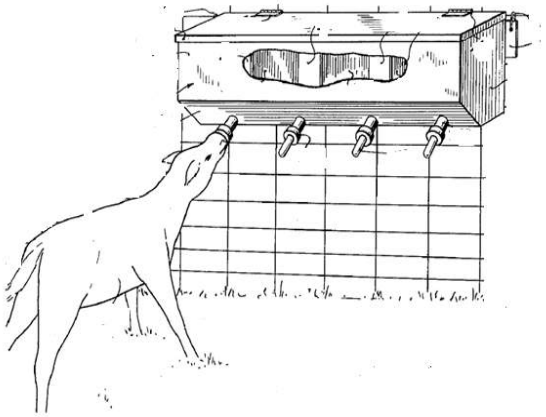
(fermentation or chemical treatment). Freezing results in virtually no loss of nutrients during storage but requires a freezer, extra handling, and daily thawing of required colostrum.

- Storage via fermentation or chemical treatment results in changes in physical characteristics, unavoidable nutrient losses, and occasional acceptability problems but is convenient and economical. Chemical preservatives are recommended for storage at warm temperatures. During storage at ambient temperatures, pH decreases as acidity increases, and total solids, protein, fat, and lactose contents of colostrum decrease.
- Total microbial numbers increase rapidly with initiation of fermentation, then level off or decline. Mold and yeast numbers continue to rise throughout storage. Some chemical additives are effective in stopping coliform growth or limiting mold and yeast growth.
- Storing colostrum at warm ambient temperatures resulted in the most rapid increase in bacteria counts, followed by intermediate rates of growth in nonpre-served refrigerated samples or preserved samples stored at ambient temperature. The most effective treatment studied was the use of potassium sorbate preservative in refrigerated samples, for which total plate count and total coliform counts dropped significantly and then remained constant during the 96-h storage period (***Stewart et al, 2005***).
- Colostrum can replace more than an equal weight of whole milk in calf-feeding programs due to its higher solids content. When colostrum is fed on an equal solids basis with whole milk, differences in calf performance are minimal. Colostrum generally does not cause scouring problems in calves. Maximal use of colostrum in calf-feeding programs is recommended **(*Foley* *and* *Otterby 1978)***

Milk feeding in weaned calves

- Once daily feeding of 3.18 kg of liquid consisting of 12 to 18% solids, fed either cold or warm, is recommended for replacement Holstein calves weaned at 21 to 28 days (***Appleman and Owen***, 1975).
- In commercial dairy farms weaning of calves within 4 days of birth is carried out. After weaning, the calf is trained to drink milk from a pail either through hollow pressure rubber tubing or a nipple.
- Farmers having a few animals allow the calves to suckle their mother.
- Milk has a high nutritive value and should be given to calves after 4 days of age. Milk is a complete feed for calves. The calf must receive sufficient milk during the first three months.

- Economical feeding on restricted milk quantity slows rate of growth which delays maturity age. Milk should be given warmed to body temperature and preferably with a trace mineral supplement to make up for its deficiency of Fe, Cu, Mg, Mn and Zn.
- Almost 60% of MC on dairy farms is inadequate, and a large number of calves are at risk of failure of passive transfer or bacterial infections, or both. Also, the data indicate that regional differences exist in colostrum quality (***Morril et al, 2012***).



Feeding Milk replacer to calves

- Ad libitum nipple feeding of milk to dairy calves can allow for increased milk intake and weight gain with no detrimental effects on intake of solid food after weaning ***(Jasper and Weary 2002)***.
- Milk repalcer is fed to calves as early as at 10 days of age to replace milk from economic point of view**.**
- Milk replacer should resemble milk more or less on broad chemical composition especially in terms of protein quantity and quality, aminoacid quantity and quality, volatile fatty acids, minerals and vitamins.
- It should have a biological value equivalent to that of milk.
- The ingredients used for preparing milk replacer should be low in crude fibre and free from any antimetabolites.
- In addition, milk repalcer may contain butyric acid to stimulate ruminal pappilary growth, citric acid as preservative and some antibiotics as additves to stimulate growth and to build up vitality and resistance against diseases.
- The repalcement of milk by milk replacer should be gradual to facilitate its acceptance and to avoid a drop in growth rate.
- Milk replacer can be fed as mash form or reconstituted in water and fed either using feeding bottles or using buckets. If reconstitution with water is done the milk replacer should be used immideatily and the water used should be potable and free from microbial load.
- Milk replacer should contain Wheat 10%, Fishmeal 12**%,** Linseed meal 40 %, Milk 13%,coconut oil 7%, Citric acid 1.5%, Molasses 10%, Mineral mixture 3%, Linseed oil 3%, Butyric acid 0.3%,

Antibiotic mixture 0.3% and Vitamin mix (A, B2, D3) 0.015%

- *L. acidophilus* supplementation for calves fed milk replacer may be beneficial during the first 2 week of life of calves (***Cruywagen et al, 1996***)

Feeding calf with Calf starter

- A standard calf starter is offered from 10th day of age to supplement the nutrients when they are raised on limited milk intake.
- The calf starter should contain 18-20 % DCP and 70-75% TDN.
- About 20-25 % DCP should be supplied through an animal protein source or skim milk powder for balancing the essential aminoacid reqiurement of pre ruminant calves which are not able to synthesize them due to their non functional rumino-reticulum.
- Calf starter is gradually introduced at the rate of 50 to 100 g / animal / day and increase upto 500 g / animal / day.
- Maize 50%, soya bean meal meal 20%, Rice bran 8%, Skim milk powder 20%, Salt 1%, Mineral mixture 2% and vitamin supplement 10 gram per 100 kg calf starter can be added to get a well balanced calf starter.

Green Feeding

Green fodder up to 250 g may be offered daily from the age of 15 days onwards to provide a stimulus for the development of rumen and as a source of carotene. Leguminous succulent roughages are preferred.

CONCLUSION

Feeding of colustrum is highly essential in first 72 hrs after birth of calf. Adequate quantity of milk has to be fed to the calf after weaning. Milk replacer and Calf starter should be properly balanced. Perfect time management in feeding of calf should be maintained to get optimum growth rate. Thereby, they will mature in time for breeding.

Scientific feeding and Hygienic shelter management will ensure the healthy calf production

Table.1. Feeding Schedule of Calves

Age (days)	Colustrum (Kg)	Milk / Milk replacer (Kg)	Calf starter	Roughage (As such basis)
1-4	1/10 of body weight	-	-	-
5-30	-	1/10 of body weight	50 - 100g/ calf	250 g
31-60	-	1/15 of body weight	150 - 250g/ calf	500 g
61-90	-	1/20-25 of body weight	250 - 500g/ calf	1000 g

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Calf Rearing: Individual And Group Housing System

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"Proper rearing of calves improves good behaviour and growth which leads to better performance in the future." Recently, many worldwide organizations have focused great emphasis and concern on the impact of animal housing in animal well-being. It is important for all producers to enhance the well-being and quality of life of dairy calves. Providing adequate nutrition, health care, animal husbandry, and management are essential. Ensuring the proper environment for each calf is equally as important. The optimal environment for housing newborn and growing dairy calves should provide physical, psychological, and behavioural comfort. The type of housing affects all these variables directly or indirectly. All variables should be considered when developing a management plan and therefore, a suitable housing system needs to be found that is most beneficial to the well-being and performance of the animal during making management plan for rearing of calves.

Basic Housing Requirements

Numerous calf housing options are available, each having advantages and disadvantages but for good housing, requirements are basically the same. Good calf housing facilities should:

- Be in a completely separate area, away from the main dairy housing barn
- Have pens that should be clean, dry, free of draught, well ventilated and amply lighted
- Have optimum temperature of 50 to 60º F and a relative humidity of 65 to 75%
- Provide convenient storage for feed, bedding and supplies
- Be constructed of durable and easily cleaned materials especially in the area where the youngest calves are housed
- Free of projections that may cause injury

Gold Standards for calves from 24 hours of life to 60 days include these goals:

1. Mortality: < 5 percent
2. Scours requiring intervention lasting at least 24 hours: < 25 percent
3. Pneumonia requiring treatment:< 10 percent
4. Growth: Double birth weight by 60 days

When selecting a calf housing system, there is need to consider climate, budget and labour constraints and individual preferences. Remember, even the very best facilities will not succeed without proper management. Some advantages and disadvantages of individual and group housing system are being described below that can be

considered while selecting any type of housing system for calves.

Individual Housing System

ADVANTAGES:

- Offer good access potential for the caretaker
- Close monitoring of each calf and observation of individual calf behaviour and health
- Allow separation of a calf from other stock and reduce spread of disease
- Provide good ventilation
- Permit ease of cleaning and sanitation
- Permit less exposure to faecal material
- Least risk of diarrhoea and respiratory disease
- Allow easier record keeping
- Have a well defined eating and resting space
- Less cross-suckling behaviour

DISADVANTAGES:

- Little opportunity for contact between calves
- Limits the extent to which the calf can behave naturally
- Growth check at weaning
- More labour intensive
- Labour intensive feeding

Recommendations for dealing with potential problems:

- ✓ Always provide milk via a teat to satisfy the motivation to suck, do not use buckets
- ✓ Provide calves with an opportunity to exercise
- ✓ Engage in normal social behaviour for some time each day
- ✓ Milk volumes should be adjusted sufficiently, particular in winter months
- ✓ Position houses to minimize environmental impacts

Group Housing System

ADVANTAGES:

- Allows early social interactions to develop skills needed for group living through play behaviour, which is important for the development of normal social responses later in life

- Provide improved access to space, allowing for more vigorous activity and play
- Easier access for mechanized cleaning
- Less labour intensive, easier management, suited to group feeding systems
- Reduces the labour associated with cleaning calf pens and calf feeding
- Less fear of other calves, novel environments

Disadvantages:

- Harder to monitor individuals
- More disease risk due to increased contact between calves
- More attention on hygiene needed to control disease
- Competition between calves
- Uneven growth rates
- More chances of developing cross-suckling behaviour.

Size of the Group Matters

Studies indicated that calves housed in large group pens had a higher risk for respiratory disease compared to calves in individual housing or small group pens. Calves housed in group pens fare better in smaller groups of 6 to 9 animals compared to 12 to 18 per group. Respiratory disease incidence was lowest in calves housed individually, intermediate in those housed in small group pens (with 3 to 8 calves), and greatest in calves housed in larger group pens (6 to 30 calves with automated feeders). A conclusion from this is that if pre-weaned calves are going to be housed in group pens, the numbers of calves per group needs to be considered.

Recommendations for dealing with potential problems:

- ✓ Incidence of disease is reduced if groups are small and also record highest gains
- ✓ Cleanliness, adequate ventilation and feeding management are considered more important than housing type for disease prevention
- ✓ It is important that calves feed using a teat (nipple bucket or bottle) rather than a bucket and are allowed to suck for an adequate time after their meal to eliminate problems with cross sucking
- ✓ Too many calves for the number of teats increases social competition and reduces intake, so keep group size small

CONCLUSION

Each type of housing system has some merits and demerits in their own way. For a calf in his whole life with any single type of housing system does not seems to be perfect in all; respect of growth, health and welfare of calves. It is concluded that individual housing can be more beneficial for early life (up to 6-8 week) that provide opportunity for accurate feeding, observation and good health whereas, group housing system with small group size and proper hygienic maintenance can be a good way for better performance and behaviour during rest of life.

Alternative Medicine Used in Anestrous Management

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Anestrus is one of the most commonly occurring reproductive problems in cattle and buffalo in India, affecting livestock productivity and economics to a great extent. It is a functional disorder of the reproductive cycle which is characterized by absence of overt signs of estrus manifested either due to lack of expression of estrus or failure of its detection. Anestrus is observed in post pubertal heifers, during pregnancy, lactation and in early postpartum period in adult animals. The condition may be associated with uterine pathology such as pyometra, fetal resorption, maceration and mummification. Expression of estrus is also influenced by seasonal changes, stress and aging. In heifers, it poses a herd problem possibly due to low plane of nutrition, stress of seasonal transition or extremes of climatic conditions. Expression of overt signs of estrus is greatly affected by heat stress in buffaloes. Modern feeding and managemet practices also accentuate the problem in commercial dairy farms. Incidence of anestrus though varies in the different management system but it is more in buffalo than the cattle, and especially during summer. Anestrus is a multicausative factors associated problem but its occurrence signals the inadequate nutrition, environmental stress, uterine pathology and improper management practices. Incidence a large variation on incidence of anestrus has been reported in literatures depending upon species, breed, parity, season, level of nutrition, management conditions, geographic environment. In general, incidence in India has been reported between 2.13 to 67.11 percent in indigenous cattle (Thakor and Patel, 2013) and 9.09 to 82.50 percent in buffaloes (Kumar et al., 2013; Thakor and Patel, 2013). The incidence of anestrus among crossbred cattle has been reported between 2.55 to 40.4 per cent (Narladkar et al., 1994) from different parts of the country. Its incidence in heifers has been reported between 12.37 to 64.66 per cent (Sinha et al., 1987). Incidence of anestrus is higher in adult cattle and buffalo than the heifers (Bharkad and Markandeya, 2003).

ECONOMIC IMPACT

High milk production and excellent fertility are desirable traits for a profitable dairy enterprise. Infertility due to cyclicity failure or anestrus has great economic

impact. Anestrus, leads to economic losses through increased intercalving interval, poor net calf crops, production loss, treatment expenses and cost of replacing mature animal with first calving heifer. There are only few reports from India pertaining to economic impact analysis due to anestrus. Pawshe et al. (2011) reported an estimated loss from anestrus around Rs.193.00 per day in cow and Kumar et al. (2013) reported Rs. 372.90 per day in buffalo. As the incidence of anestrus in India has been reported high, the above figures show great economic impact at country level.

TREATMENT (PLANT BASED HEAT INDUCER)

Plants have been used for the treatment of animals since long back. Plants synthesize varieties of phytochemicals such as alkaloids, glycosides, terpenes and tannins (secondary metabolites) as a part of their normal metabolic activity and many of these have therapeutic actions when consumed by animals. Many plants are rich source of vitamins and minerals whereas some have estrogenic property which is useful in restoration of cyclicity in anestrus animals. Almost all the parts of plant such as seeds, berries, roots, leaves, bark and flowers have been used as therapeutic agents either directly (crude drugs) or their active principles, after separation though various chemical process.

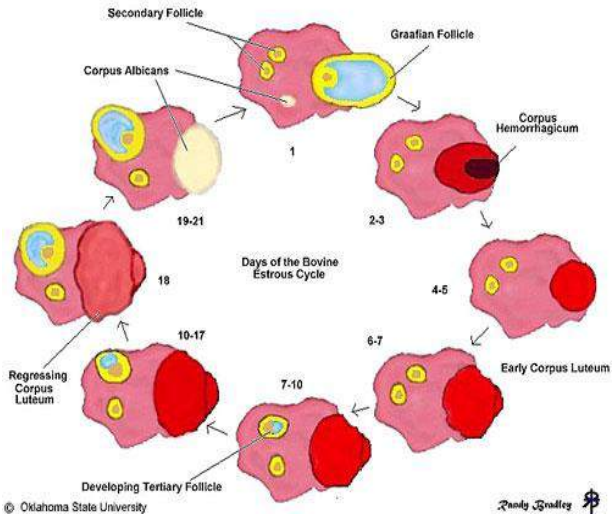
Mixed preparation

- Black pepper (10 grains) and vanghuchi (20-25 grains) is mixed together and given twice a day at the interval of 6-8 hours for 1-2 days for treatment of anoestrus.

- Kabir et al. (2001) reported 50% estrus induction in anestrus buffaloes using mixture of Abroma augusta (root) and Nigella sativa (seed) in 2:1 ratio.
- Rajkumar et al. (2008) reported higher success rate in anestrus cattle i.e. 83.33 and 66.66% using Methi seed (@ 200g/day/cow for 20 days) and bark of Ashoka tree (@ 50g/day/cow for 20 days), respectively.
- A leaves of silk cotton tree are powdered together with fermented boiled rice water and the extract is administered to cows orally as a remedy for reproductive problems. Approximately 500 ml, three times a day for 3 consecutive days (Ranjan and Sethuraman, 1997).
- Indigenous herbal preparations such as Prajana HS (Indian Herbs), Janova (Dabur), Sajani (Sarabhai), Heat up (Century) Heat raj (Ranjan), Fertivet (Ar Ex Labs) and Aloes compounds (Alarsar) are commercially available and effective in restoration cyclicity with good success rates (Hussain et al., 2009). These formulations are potent combinations of herbs, formulated to induce ovarian activity

Table No. 1: Plant species used in Anestrous Management

Botanical name /Common name	Parts used	Use
Aegele marmelos **(Bale*)***	Leaves	Fed over 6-7 days to animals to overcome anoestrus problem
Amomum subulatum + Foeniculum vulgare + Trachyspermum ammi + Papever hybridum	Fruits+ Seed+ Seed +petals	Mix 100g, 250g, 50g, and 250g, respectively; administer per os as one dose and continue treatment for 3 days
Bambuseae **(Bamboo)**	Leaves	Fed to cattle to bring it to the regular heat
Carica papaya (**Papaya)**	Fruits	Fed 2-3 kg unripe papaya fruits once a day for 4-5 days to bring animal to the regular heat
Corchorus capsularis **(White jute*)*** ***Corcorrus olitorius*** **(Tossa jute*)*** **Jute plant**	Leaves	Fed 2-2.5 kg leaves to animal to bring it to the regular heat
Cucumis sativus **(Cucumber)**	Leaves	Fed to the animal to bring it to the regular heat
Cuminum cyminum (sufaid zeer)	Seeds	Administer per os 125g seeds as such for 4 days
Ficus religiosa (Peepal)	Leaves	Boiled 500g leaves in 2 L water; on remaining 250ml as per os for 3 days.
Leptadenia reticulate, Asparagus racemosus, Couroupita guianesis	Leaves	Fed over 6-7 days to animals to overcome anoestrus problem
Mann tree	(leaves):	Fed 15-20 kg leaves can overcome anoestrus condition
Morus indica (shehtoot)	Leaves	1 kg leaves are orally fed for 5days
Pergularia daemia **(Dudheli)**	Pods	Fed Pods to animal to overcome anoestrus problem
Rosa indica (guab)	Flower	Boiled 250g flowers in 1 L water, add 1 kg jiggery; administer per os for 5 days
Semecarpus anacardium **(Bbhilama*)***	seeds or fruits	fed three to four seeds or fruits to animal for very day for 3-4 day when it is not coming into heat or fails to conceive successfully
Trigonella foenum-graecum **(Fenugreek)**	Seeds	Fed to the cow can overcome the Anoestrus
Zizyphus nummularis (bairy)	Leaves	Feed 1 kg leaves for 8 days



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Utero ovarian massage is the oldest, simplest, cheapest and effective method to induce estrus in anestrus cattle and buffaloes (Rahawy, 2009; Mwaanga et al., 2010). Estrus induction in cattle and buffalo varies between 40 to 80% following utero ovarian massage daily/on alternate day/weekly for 3 4 weeks (Mwaanga et al., 2004; Naidu et al., 2009; Gupta et al., 2011). Probable mechanism includes: activation of intrinsic intra ovarian factors; enhancement of blood circulation to the ovaries and uterus that increases the availability of hormones and growth factors; stimulation of local oxytocin production by the ovaries which consequently influence local blood circulation and luteolysis, if CL is present (Mwaanga et al., 2010).

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Review Article

Characteristics of Indigenous Breeds of Buffalo: An Overview

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The domestic or water buffalo (*Bubalus bubalis*) belong to the family *bovidae*, sub-family *bovinae*, genus *bubalis* and species *arni* or wild Indian buffalo. Buffalo are believed to have been domesticated around 5000 years ago in the Indus Valley. The water buffalo can mainly be classified as River (Chromosome no., 2n=50) and swamp type (Chromosome no., 2n=48). The domestication of swamp buffalo took place independently in China about 1000 years later. The movement of buffalo to other countries both east and westwards has occurred from these two countries. Some of the well-known dairy breeds of buffalo found in India and Pakistan are Murrah, Nili-Ravi, Kundi, Surti, Jaffarabadi, Bhadawari, Mehsana, Godawari and Pandharpuri. The swamp buffalo are concentrated mainly in south east China, Myanmar, Malaysia, Laos, Cambodia, Thailand, Indonesia, Philippine, and Vietnam. The skin colour is gray, dark gray to state blue. White animals occur frequently. Animals have swept back horns and are similar in appearance across the countries except the size. The horns grow laterally and horizontally in young animals and curve round in a semi circle as the animals gets older. Animals are massively built, heavy bodied with large belly. The forehead is flat; orbits are prominent with a short face and wide muzzle. They weight from 300 to 400 kg when fully grown. Swamp buffalo are primarily used as work animal in paddy cultivation, for pulling carts and hauling timber in jungles. Milk yield is 1-2 kg per day.

BREEDS: On the basis of regions the well defined buffalo breeds are:

(A) MURRAH GROUP:

Murrah:

Most important breed of buffaloes whose home is Rohtak, Hisar and Sind of Haryana, Nabha and Patiala districts of Punjab and southern parts of Delhi state. Otherwise called as Delhi, Kundi and Kali. The colour is usually jet black with white markings on tail and face and extremities sometimes found. Tightly curved horn is an important character of this breed. Most efficient milk and butter fat producers in India. Butter fat content is 7.83%. Average lactation yield is varying from 1500 to 2500 kgs per

lactation. Also used for the grading up of inferior local buffaloes. The intercalving period is 450 to 500 days. The age at first calving is 45 to 50 months in villages but in good herds it is 36 to 40 months.

1. **Nili Ravi:**

Originated around the river Ravi. This breed is found in Sutlej valley in Ferozpur district of Punjab and in the Sahiwal (Pakistan) of undivided India. The peculiarity of the breed is the wall eyes. Head is small, elongated, bulging at top and depressed between eyes. Horns are very small and tightly coiled. Bullocks are good for heavy trotting work. The milk yield is 1500-1850 kgs per lactation. The intercalving period is 500 to 550 days. The age at first calving is 45 to 50 months. The bullocks are good for heavy trotting work.

2. **Kundi:**

Kundhi Female Kundhi Male

The word kundi means fish-hook in sindhi language. The Kundi breed is of the milk type. It is found in Dadu, Hyderabad, Karachi, Larkana, Nawabshah, Sanghar and Thatta districts in Sind Province. The color is solid black. The average weight at maturity for the male is 600 kg and 375 kg for the female. They are massive animals. The horns are small and spirally twisted and hence the name "Kundi". The fore head is slightly prominent, the face hollow and eyes are small. Mammary gland are capacious with prominent milk vien; teats are squarely placed. The udder is large and strong and the longevity of production is 'good'. Kundi buffaloes are smaller than Nili-Ravi with adult weight of 320 to 450 kg.

4. **Godavari:**

Godavari is a result of crossing of native buffaloes with Murrah bulls. The home tract is Godavari and Krishna deltaic area. The animals are of medium stature with compact body. The colour is predominantly black with a sparse coat of coarse brown hair. Godavari buffaloes are reputed for high fat with daily average milk yield of 5-8 litres and lactation yield of 1200-1500 litres. The animals breed regularly and have a short calving interval compared to Murrah. They are hardy and

possess good resistance against majority of prevailing diseases.

(B) GUJARAT GROUP:

1. 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.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 (8-12per cent). The bullocks are good for light work.

2.Jaffrabadi:

The breeding tract of this breed is Gir forests, Kutch and Jamnagar districts of Gujarat. This is the heaviest Indian breed of buffalo. The horns are heavy, inclined to droop at each side of the neck and then turning up at point (drooping horns). The udder is well developed with funnel shaped teats. The average milk yield is 1000 to 1200 kgs per lactation. The bullocks are heavy and used for ploughing and carting. These animals are mostly maintained by traditional breeders called Maldharis, who are nomads. The bullocks are heavy and are used for ploughing and carting.

3. Mehsana:

Mehsana is a dairy breed of buffalo found in Mehsana, Sabarkanda and Banaskanta districts in Gujarat and adjoining Maharashtra state. The breed is evolved out of crossbreeding between the Surti and the Murrah. Body is longer than Murrah but limbs are lighter. The horns are less curved than in Murrah and are irregular. Bullocks are good for heavy work. The milk yield is 1200-1500 kgs per lactation. The breed is supposed to have good persistency. The intercalving period ranges between 450 to 550 days. The bullocks are good for heavy work but rather slow.

4.Banni:

Banni Buffalo breed was recognized as 11th buffalo breed of India by Breed Registration Committee, ICAR, New Delhi. The breed is originated from the Banni area of kachchh, which is a part of Kachchh district of Gujarat. Purebred animals prevalent in Bhuj, Nakhatrana, Anjar, Bhaahau, Lakhpat, Rapar and Khavda talukas, are heavily size with typical double and vertical coiling of the horn. The ***"sui-genesis"*** germplasm of kachchh i.e. ***"Banni buffaloes"*** are maintained by maldharis under typically and locally adapted extensive production system in its breeding tract. The body coat colour is black (90.09%) and copper (9.90%), whereas muzzle and eyelids are either black or brown. Horns orientation is vertical, inverted double coiling in 31.20% and vertical, inverted single coiling in 68.80% animals. Eyes are prominent black and bright. The colour pattern of the switch of tail comprises (67.35%) white and (32.65%) black and length of the tail is 88.39+-0.48cm. Body is Medium to large, compact and generally covered with hairs. Dewlap is absent and naval flap is medium. Head is Wide with slight depression in the middle and no slope towards base of the horns. Face is Comparatively elongated and straight with wide muzzle. Neck is Medium and thin without skin folds over the region. Ear orientation is horizontal in majority of animals and length of ear is 29.30+-0.08cm. Udder is Well developed, round in shape and squarely placed. The hind and fore quarters are uniformly well developed, whereas typically whole udder looks like four equal divisions with teats well attached to each quarter. Teats are Majority of animals have conical teats with round and pointed tips.

(C) UTTAR PRADESH GROUP:

1. Bhadawari:

Home tract of this breed is Agra and Etawah district of Uttar Pradesh and Gwalior district of Madhya Pradesh. Medium sized buffalo. The body is usually light or copper coloured is a peculiarity of this breed. Eye lids are generally copper or light brown colour. Two white lines 'Chevron' are present at the lower side of the neck similar to that of Surti buffaloes. The average milk yield is 800 to 1000 kgs per lactation. The bullocks are good draught animal with high heat tolerance. The fat content of milk varies from 6 to 12.5 per cent. This breed is an efficient converter of coarse feed into butterfat and is known for its high butter fat content.

2. Tarai:

The breed gets the name from the Tarai area of U.P., Where it is mostly found between Tanakpur and Ramnagar. The breed is native of hilly area. They are Frequently crossed with Murrah bulls. It has a moderate body, slightly convex head with prominent nasal bones. Horns are longs and flat with coils, bending backwards and upwards and upwards having pointed tips. The eyes are rather small but ears are long and coarse. Legs are short but strong. The tail is long, reaching below the hocks. The colour of the skin varies from black to brown. The switch of the tail is white. The breed is poor regarding milk production, which may be as low as 2-3 kg daily. Males are efficient draught animals used for agricultural operations as powers including transport.

(D) CENTRAL INDIA GROUP:

1. Nagpuri:

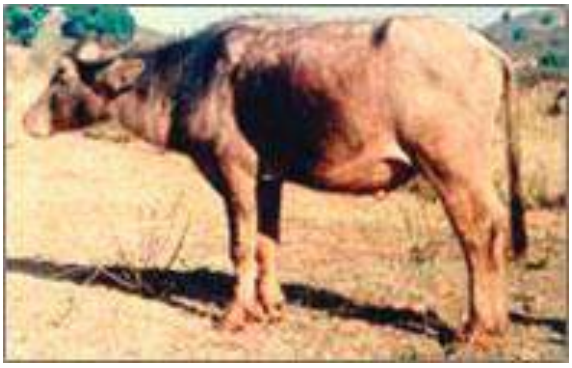
This breed is also called as Elitchpuri or Barari. The breeding tract of this breed is Nagpur, Akola and Amrawati districts of Maharashtra. These are black coloured animal with white patches on face, legs and tail. The horns are long, flat and curved, bending backward on each side of the back. (Swaord 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 and intercalving period is 450 to 550 days. The bullocks are good for heavy trotting work but slow in movement.

2. Pandharpuri:

The name Pandharpuri is from the town Pandharpur in Solapur district which is the home range of these buffalo. They are found in Solapur, Kolhapur and Sangli districts of Maharashtra. The majority of the breed are black with white markings found on the forehead , legs and switch of tail. It is medium sized with average body weight of 450-470 kg. Animal having long narrow face, very prominent and straight nasal bone, comparatively narrow frontal bone and long compact body. The udder is compact, trough shaped with cylindrical

teats. The head is long, narrow with prominent nasal bone and the horizontal ears. Typical characteristic of this breed is its horns which are very long, extending beyond shoulder blade, sometimes up to pin bones, curved backward, upward and usually twisted outwards, which measure from 45-50 cm upto 1-1.5 m of length. The breed is famous for its high reproductive ability, producing a calf every 12 months. Under average management conditions and hot -dry climate these buffaloes yield 6-7 liters of milk per day; however under good management they are reported to yield up to 15 lit of milk per day.

3. **Manda:**



The animals are bred in the hills above Parlakimedi and Mandasa on the borders of Orissa and Andhra Pradesh. Basically the breed is reared in areas of thick forest usually on natural herbage and brought down to the plains for sale. The general colour is brown or grey with yellowish tufts of hair on the knees and fetlocks, and the switch is yellowish white. The breed is a medium sized animal. The eyes are sharp with a broad red margin around the lids. The horns are broad and semi-circular extending backward and well developed chest. Milk yield is satisfactory. Males are hardly and like a bullock can work in the hot sun. It can draw a load of about a ton but at a slow pace.

4. Jerangi:

This breed of buffalo is widely distributed in Jerangi hills of Orissa and Northern parts of Visakhapatnam and west of Ganjam in Andhra Pradesh. One of the dwarf breeds of buffalo and its height does not exceed four feet. Horns are conical and small and run backward, body colour is black. Not that much good in milk production but are useful animals for ploughing in water-logged paddy fields.

5. Kalahandi / Peddakimedi:

Kalahandi buffaloes are dual type; used for milk and draught purpose in Kalahandi and Rayagada districts of Odisha. Animals are medium sized; having long, strong, half

circled horns with broad base; and are excellent in heat and drought tolerance. The colour is grey or ash grey with white switch of medium length tail. The fore head is slightly protuberant. Due to light colour it tolerates sun heat better than dark colour buffaloes. Milk yield is quite satisfactory.

6. **Sambalpuri / Kimedi / Gowdoo :**

The home tract of this breed is controversial. Originally it was known to be habitat of Sambalpur area of Orissa, later on it has been suggested that the main habitat of this breed is around Bilaspur district of M.P. wherefrom calves are brought by "gowdoo"(Herdman) to Sambalpur area. Animals are large and powerful having long, narrow barrel and prominent fore-head. Body and coat is generally black but it varies to brown and ash grey. Males are very active and good for drought purposes which are affected by high atmosphere temperature. Females breed regularly and produce milk satisfactorily. Average milk yield varies from 2270 to 2720 kg in 340 to 370 days with a daily average of over 7 kg in good milkers.

(E) SOUTH INDIA GROUP:

1. Toda:

This buffalo is named after an ancient tribe, Toda of Nilgiris Hills of south India and it is a semi-wild breed. The predominate coat colours are fawn and ash-grey. Thick hair coat is found all over the body. They are gregarious in nature. The body is long and deep and the chest is deep. The legs are short and strong. The horns are set wide apart curving inward, outward and forward forming a characteristic crescent shape. Toda buffaloes are good milkers yielding from 4.4 to 8.8 litres of very rich milk. The average milk yield is 500 kg per lactation with high fat content of 8%.

2. South Kanara:

South Kanara buffaloes are medium built animals distributed in South Kanara region around Mangalore and Udupi on the west coast of India. The presence of buffaloes in

its original habitant has decreased substantially while more such animals are found in the adjoining Shimoga districts. The body coat colour varies from brown to silver grey and black. Horns are flat, corrugated and curved projecting backward, sideward and upward at the neck region. Tail is fairly long, thin and flexible ending in a black switch. South kanara buffaloes are well built and medium sized animals. Head is fairly long with broad forehead. Neck is long with moderately thick dewlap. Ears are moderately long and erect. Udder is moderately developed. Teats are medium sized and squarely placed behind the hind legs. South kanara buffaloes are moderate milk yielders and normally give milk ranging from 2 to 7 liters per day. There are animals in villages with a peak yield of more than 10 litres per day. The length of lactation varied between 210 to more than 360 days. The lactation milk yield varied from 420 to 2520 litres. The age at first calving and calving interval varied varied between 30 to 60 months and 12 to 36 months respectively.

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Major Histocompatibility Complex of Cattle and Their Role in Disease Resistance

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The term major histocompatibility complex derives from research in transplantation during mid-twentieth century. Studies of tumour graft fate in mice and skin transplants between identical twins indicated that genetic factors were involved in allograft rejection. These experiments provided insight into the rules governing the acceptance or rejection of tissues, when tissues were transplanted between different members of same species. Researchers earlier interpreted that rapid rejection of transplants were determined by a single gene, so earlier they named it, the major histocompatibility gene. But later studies indicated that a set of closely linked genes are responsible so named as the major histocompatibility complex. The genetic region, now known as the major histocompatibility complex (MHC), was first defined in mice by Gorer. Antibodies, T cell receptors and the major histocompatibility complex (MHC) are defining components of the adaptive immune system with the recognition and binding of signature pathogenic sequences by specific lymphocyte receptors integral to initiating an adaptive immune response. The ability of the immune system to distinguish self from non-self is reliant on a process named MHC restriction. During development of an individual's immune system, immature T cells within the thymus are exposed to self peptide- MHC complexes in a process known as clonal selection. Almost, half of the loci encoded on the gene dense MHC region are dedicated to immune functions (Kelley *et al*., 2005). The MHC region is subdivided into three classes (Class I, Class II and Class III) based upon gene product function (Rhodes & Trowsdale, 1999).

The key functions of MHC molecules are twofold:

(a) To bind peptides those are produced when proteins are catabolised-processes-inside cells of the host.
(b) To present peptides to T cells with the appropriate TCR (T cell receptor).

BOVINE MHC (*BoLA*)

Genetic organisation

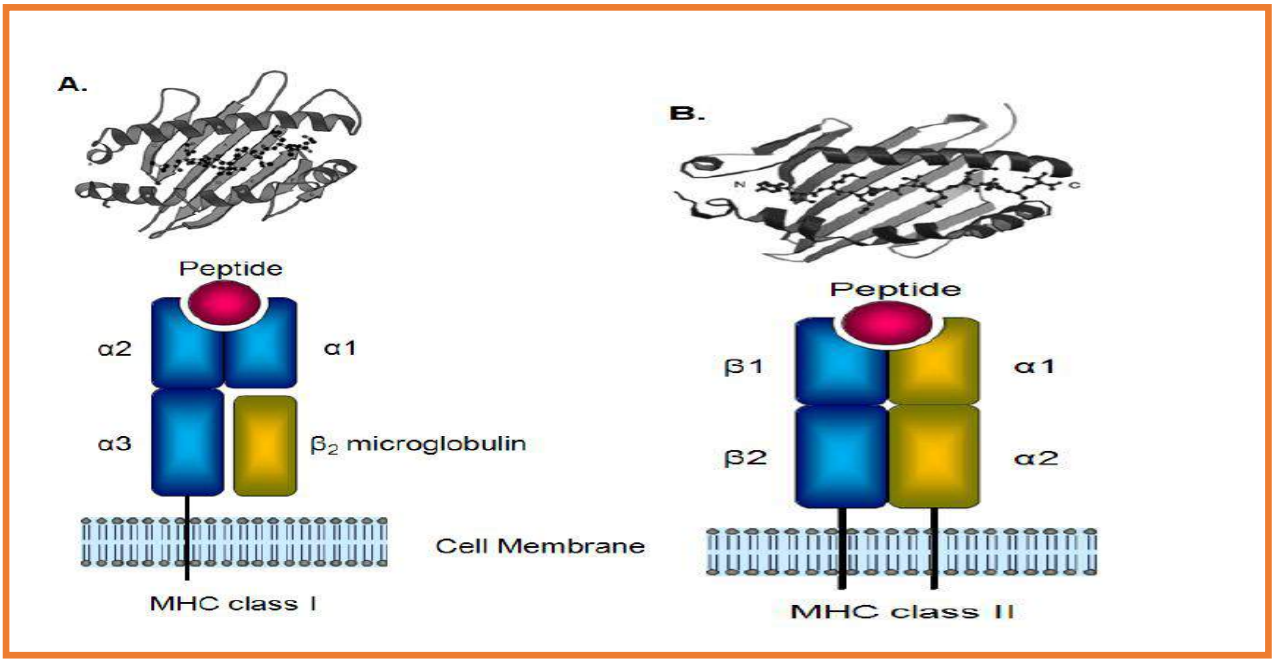
Group of genes on cattle chromosome 23 is called MHC. MHC of *Bos Taurus* and *Bos indicus* is called BoLA (Bovine Leucocyte Antigen System). More than 30 genes mapped to bovine BTA 23 (*Bos Taurus* autosome 23). All animals possess a MHC molecule in their genome which are named differently in different mammals. In cattle the MHC gene i.e. BoLA is a misnomer as Bovine represents a wide range of genera (six) under sub-family *Bovinae*. Though BoLA is a misnomer yet applied only for cattle and not for any

other species of genus Bos. BoLA system is located on two distinct segments of cattle chromosome 23. This organisation distinguishes BoLA from the MHC of rodents and primates. Unlike humans and mice, the bovine class II region is subdivided into class IIa and class IIb which are separated by a physical recombination distance of 17 centimorgans (Andersson & Rask 1988). BoLA class IIa, class III and class I regions map to BTA 23q22 approximately 35 centimorgans (Cm) from the centormere. Class IIb region is located 15-30 cM proximal to the centromere and the class I region spans about 770-1650 kb and contains 10-20 different genes.

A Gene order comparison between humans, mice and cattle indicates that the class II division is a result of single large chromosomal inversion (Band et al., 1998). MHC molecules are membrane bound glycoprotein Bovine MHC i.e. BoLA has three classes: Class I molecule, Class II molecules and Class III molecules. Class I and Class II products are cell associated molecules present on all nucleated cells in higher animals, involved in induction and regulation of immune response.

***BoLA* class I molecules:** These are heterodimers, consist of α (heavy) chain associated non-covalently with much smaller β_2 – micro-globulin molecule and expressed on all nucleated cells. They present peptides to $CD8^+$ T lymphocytes which kill virus infected and neoplastic cells.

***BoLA* class II molecules:** These expressed on 'professional' antigen presenting cells (APCs) like Dendritic cells and Macrophages. They consist of two polypeptides α- chain and β-chain. Class II genes are distributed to two regions: IIa and IIb with -17% recombination frequency. DRA, DRB, DQA and DQB genes are located in IIa region. And DO (DOB), DY (DYA and DYB) and DI (DIB) genes are located in IIb region. DRB has three genes: DRB 1; DRB 2; DRB 3; of these DRB 3 has high level of expression. Class II molecules present small antigenic peptides in their antigen binding groove, which is encoded by second exon of the gene, has high degree of polymorphism. This polymorphism determines the capability of MHC molecules to bind to a wide range of peptides. Immune response against antigen depends upon the



effective binding of antigen to the peptide binding motif of MHC class II molecules. Genes encoding these motifs are highly polymorphic and DRB 3 genes are associated with general health, disease resistance and disease susceptibility.

***BoLA* class III molecules:** They are the multifunctional molecules and involve in functions from cell (Cytotoxic: Tumor Necrosis Factor) to enzymatic activities. Three dimensional structures of MHC molecules reveal precisely how the enormous polymorphism of MHC molecules is related to genetic control of immune response and disease susceptibility. Study of MHC of cattle promises manipulation of immune responses for improving resistance of infectious diseases.

Fig (A) and (B) showing MHC class-I and MHC class-II molecules

MHC and Diseases

The function of MHC molecules is to regulate immune system by presenting antigens to immune system. An immune response can only take place if the antigen binds to the groove of an MHC molecules, it will not stimulate an immune response. So these genes influence resistance and/or susceptibility to diseases where immune response plays a significant role. Association between types of MHC allele and disease resistance has been studied in several species. Few examples of association between certain BoLA alleles and disease resistance/susceptibility in cattle are as follows:

- Bovine leucosis
- Dermatophilosis
- Tick infestation
- Helminthiasis
- Mastitis
- Brucellosis

BOVINE LEUCOSIS

A viral disease caused by bovine leukaemia virus (BLV). Cattle infected with BLV and suffering persistent lymphocytosis (PL) have reduced milk and fat yields. In a herd of shorthorn cattle resistance and susceptibility was found to be associated with alternative BoLA haplotypes defined by class-I serology. An association was found between development of PL in cattle and DRB2. Resistance to persistent lymphocytosis is associated with presence of Glu-Arg residues in the antigen binding site of DRB3 at position 70 & 71. Several alternative DRB3 types have been found to be associated with resistance/susceptibility to PL in American HF and Black Pied cattle.

Dermatophilosis

A skin infection with varying severity caused by bacterium *Dermatophilus congolensis.* The disease occurs sporadically throughout the world in many species including humans. Severest form in cattle occurs in tropical humid regions e.g. West Africa and the Caribbean. West Africa cattle breed N'Dama revealed resistance to Dermatophilosis. Polymorphism in BoLA-DRB3 exon 2 gene(s) in Brahman cattle of Martinique revealed a strong association with both resistance and susceptibility to Dermatophilosis. In the same population a weaker, but significant, association with BoLA-A8 class-I type was also found. Genes for resistance to Dermatophilosis occur in MHC region of Brahman cattle. But it is not clear whether this gene (s) is a class-I gene and/or class-II gene or another linked gene (s), segregation studies should clarify this.

Tick infestation

Tick infestation is caused by several tick species of major genera such as:

- *Boophilus microplus*
- *B. decoloratus*
- *Amblyomma americanum*
- *A. hebraeum*
- *A. variegatum*
- *Rhipicephalus appendiculatus*

Cattle tick resistance is measured by overall visual assessment of tick burden most commonly as Tick count and Effects on ticks:

- Tick survival
- Weight of tick
- Fecundity
- Effect on tick population growth rate

When cattle are challenged by multiple tick species, resistance is developed to all of them. Proportion of ticks of different species tends to be same on susceptible and resistance animals. Most common observation on tick resistance irrespective of the species is relatively high resistance of *B. indicus* in comparison with *B. taurus*. Crossbred have generally been intermediate in tick resistance in comparison with pure *B. indicus* and *B. taurus*. Exception to the general rule that *B. indicus* is more tick resistant than *B. Taurus* is N'Dama cattle (*B. taurus*) of west Africa which has highest level of resistance. MHC class-I typing of cattle for resistance and/or susceptibility to *Boophilus microplus* revealed weak associations. Overall history of exposure to tick is most important factor in determining levels of resistance irrespective of the cattle is not known, different mechanism may operate in different circumstances.

Helminthiasis

Helminthiasis the gasterointestinal nematode and trematode infections is a worldwide problem. In economic terms, productivity losses due to helminthiasis are significant. Risk of development of resistance to the anthelminthic drugs in target parasite is high and real. Breed differences in helminthiasis resistance has been seen in field conditions in Zambia. N'Dama cattle sheds lower number of faecal eggs during high challenge period and carry lower worm burden than zebu cattle. Significant heritabilities for faecal egg output were reported in large herd study. Weak association was found between BoLA class-I type and faecal egg outptut. Little is known about mechanism of genetic control of helminthiasis resistance.

Mastitis

Due to economic importance of mastitis, the genetic basis of resistance/susceptibility has received considerable attention. The heritability of mastitis resistance and susceptibility has been established in many cattle breeds. Somatic cell score in milk has high correlation with mastitis occurrence. In Molecular marker studies, association of MHC class-I and class-II types with mastitis at population and breed level is most focused. However, some studies have been reported with no association with mastitis. The large progeny-test studies also indicate a role of genes in MHC region for mastitis resistance. It is not clear whether genes other than MHC such as genes controlling morphological features also contribute to mastitis resistance. Weak association was reported between immune response and mastitis resistance. Variation in mastitis

resistance and/or susceptibility is polygenic in nature because it is influenced by genes that also control IgG_2 isotype, Non-MHC leucoyte antigens or genes linked to these.

BRUCELLOSIS

There are evidences of natural resistance to Brucellosis in East African shorthorn zebu cattle. Breeding experiments confirmed variation in heritability of Brucellosis resistance. When males and females were challenged with *Brucella abortus* S2308 during mid gestation resistance cows did not abort and no Brucella organisms were recoverd from cows and calves. There are evidences that the resistance to Brucellosis is partly under the control of the bovine *Nrampl* gene but the survey of a variety of bovid species and the mode of inheritance in cattle suggest that the other genes are also involved.

CONCLUSION

The MHC of farm animals is an important region of the genome for immune responsiveness and disease resistance.Understanding how MHC polymorphism contributes to disease resistance and susceptibility, and to the development of effective immune responses, is a major goal of livestock research, requiring interdisciplinary efforts at the genomic, molecular, biochemical and immunological levels. Study of the major histocompatibility complex assumes importance because of the critical role it plays in the immune system of the animal. The molecular analysis and fine mapping of disease associations will probably play a central role in animal genetics and veterinary medicine for many years to come. The increasing resistance of pathogenic microorganisms to antibiotics and other drugs underlines the importance of understanding the molecular genetics bases underlying the resistance to infectious diseases. The extensive structural polymorphism of MHC molecules is responsible for the differences among individuals in immune response to infectious agents. This high degree of polymorphism observed may help in identification of superior haplotypes for disease resistance.

Climate Change And Integrated Pest Management

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Impacts of Climate Change on Agricultural Pests

In the last years, the permanent concern regarding the study of pest attack dynamics related to the evolution of regional agro ecological constituents has developed the observation of the impact caused by the climate changes in the cereal crop entomocenoses. Climate warming, the settlement of extremely hot periods, draught and heat during spring and summer months have been severe ecological factors which induced changes in species structure, facilitating the growth of populations belonging to a more narrow spectrum of problem-arising species which have become dominant and dangerous due to the number increases, even number bursts, and due to local invasions and powerful attacks.Losses from pests are most severe in the subtropics and tropics because of warmer temperatures; longer growing seasons, and, in some regions, year-round production that creates favorable conditions for pests, combined with a generally low capacity to manage pre- and post-harvest pests. An increase in extreme climate events, changes in moisture conditions, temperature rise, and elevated CO_2 concentrations are expected to magnify pest pressure on agricultural systems through:

- Range expansion of existing pests and invasion by new pests.
- Accelerated pest development leading to more pest cycles per season.
- Disruption of the temporal and geographical synchronization of pests and beneficial insects that increase risks of pest outbreaks.
- Promotion of minor pests to primary pests brought about by reduction in host tolerance and changes in landscape characteristics and land-use practices.
- Increased damage potential from invasive alien species.
- Narrowing of current pest management options, including nonchemical means such as host-plant resistance breeding and biological control.

Effects on Integrated Pest Management

IPM is an essential component in many of the world's crop production systems, encompassing a knowledge-intensive set of practices that rely on reasonably predictable parameters of seasonal climate conditions to determine economic thresholds for pest populations. Climate change could impair the reliability of current IPM strategies, requiring the dedication of additional resources to develop new knowledge systems and appropriate measures to counter new

pests or the intensification of existing ones. Potential effects of climate change on management practices include the following:

- Host-plant resistance may be compromised by high ambient temperatures that trigger deactivation of crop host resistance genes, and by host exposure to a greater number of pest lifecycles per growing season.
- Loss of crop wild relatives (CWR) could reduce the scope for replenishing new genes in host-plant resistance breeding programs.
- Increased seasonal climate variability and changes in humidity and temperature have the potential to disrupt enemy-herbivore dynamics, which are important for biological control.
- Loss of soil organic matter and increased rates of soil erosion could reduce the capacity of microbial populations to biologically control soil borne pests and diseases.
- Pesticides could become less effective or persistent under conditions of warming soils, increased rainfall, and CO_2 stimulation of weed biomass. Higher rates of pesticide usage disrupt natural biological control, cause secondary pest outbreaks, degrade the environment, and increase selection pressure for pesticide-resistant populations.

How to overcome this problem?

Quantitative information about future risks of pest damage from climate change is needed in order to determine where to invest resources in technology development and capacity building for pest surveillance and management. The foremost need is to gain basic quantitative information concerning which cropping systems could be vulnerable to increased pest pressure from climate change, such as implications of increased pest damage in food-insecure regions and how that vulnerability could occur (e.g. range expansion of existing pests, potential increase in number of pest cycles per season, and invasion of new pests). A comprehensive risk assessment by experts, using a common framework, is needed given the significant knowledge gaps that exist in assessing risks of pest damage under climate change. The system wide IPM program of the Consultative Group on International Agricultural Research (CGIAR) would be a logical entity to develop such a framework.

Pest–climate simulation models are an important component of such a risk assessment. The development of temperature and moisture-based simulation models can help identify where shifts in pest range or intensification of pest damage are possible with climate change and where adaptation measures could be required. For example, temperature models developed by the International Potato Center for the highly invasive potato tuber moth (PTM) revealed that the PTM range could shift about 400 to 800 kilometers north in the northern hemisphere and several 100 meters in altitude in tropical mountainous regions with temperature increases of 2°C to 3°C, and that moth activity and number of lifecycles would increase in its present range.

The elaboration of pest integrated management requires several stages of complex researches:

1. The inventory of damaging species, the understanding of regional characteristics of pest biology, ecology and control.
2. The evaluation of population dynamics under regional agro ecological conditions, including climate factors, crop pheonological development, the dynamics of entomophag natural reserve.
3. The monitorization of pest apparison and attack and the interactions with auxiliary species.
4. Planning preventive measures and control strategies which comprise several aspects regarding:

- Cropping regionally adapted, tolerant, highly yielding, damage-compensating varieties.
- Sowing during optimal time, protective against pest attack and the first dangerous invasions, and also important to provide starting rate and crop initial vegetative strength.
- The agro technical methods which mechanically diminish a large proportion of pest biological reserve (crop rotation, soil tillage, ploughing, disking, destroying volunteer herbs etc.
- Phytosanitary methods of disease and weed integrated control, balanced fertilization crucial for plant healthy growing in their fight against phytophagous insects.
- Protection, use and development of the natural entomophags reserve through conservation of auxiliary species and plant biodiversity, special development of eco marginal areas including trees, shrubs, herbs, their protection against herbicides etc.
- The use of nonpolluting-selective biotechnologies such as: cultivation of new resistant breeds and varieties, controlled parasites and predators breeding and release, placing of pheromone traps etc.
- Caution in implementing only phytosanitarily certified, nonpolluting control measures into the system that do not cause negative effects on the agro ecosystem balance and stability.

Rice moth (Corcyra Cephalonica Stainton): An Efficient Factitious Host For Mass of Bioagents

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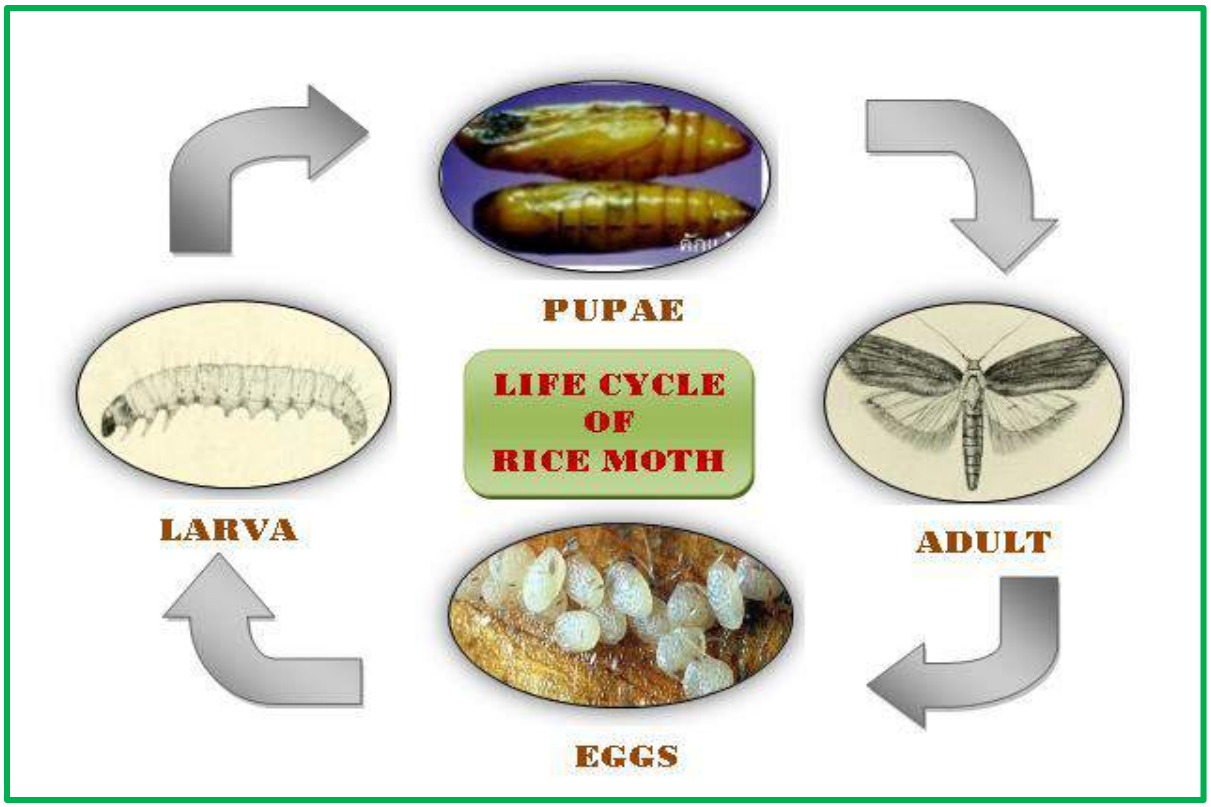
Corcyra cephalonica (Stainton), popularly known as the "Rice meal moth" or the "Flour moth", belongs to family Pyralidae of order Lepidoptera. The earlier reference of this insect was made by Stainton (1866), who provisionally named it *Melissoblaptes cephalonica* giving a brief description. Later a new genus, *Corcyra* was erected by Rogonot (1885) to accommodate this insect, the name being derived from the ancient name of "Corfu", where it was presumed to have been imported into England. According to Chittenden (1919), though *Corcyra* is known to occur in many parts of Europe, Asia and America, it is not a true cosmopolitan insect. This view cannot be readily accepted, since none of the so called cosmopolitan insects, as it was found ever in all parts of the world mainly because of lack of suitable environmental condition for a continued existence. According to Durant and Beveridge (1913), *Corcyra* is apparently of eastern origin and has been introduced into Europe and elsewhere by the rice trade. The rearing of *C. cephalonica* on different natural diet is potentially importance for the nutritional quality of host eggs and the survival of *Trichogramma* and other eggs parasitoids released into the environment as biological control agents (Hunter, 2003). It has been reported that *C. cephalonica* have a shorter development time on millet than on sorghum (Russell *et al.*, 1980); and a shorter development time on maize than on cocoa (Mbata, 1989). In this report, we worked on the hypothesis that the quality of *C. cephalonica eggs* is affected by the type of food materials used to feed the host larvae. Such result would be an example of a "bottom-up cascade" of ecological effects.

BIOLOGY

The adult of rice meal moth is grey in colour and does not feed. The mated female lays about 100 to 200 eggs near food sources. Eggs hatch in 4-10 days. The larva constructs a feeding tube gallery, consisting of silken web and food particles to stay feeds and grow inside it. When they are fully-grown, they form dense white cocoons to pupate. Pupae are usually found in the food or they may be found between pallets and sacks. Adult emerges from pupae within four to eight weeks and repeat their life cycle.



Importance of rice moth as a host

Mass rearing of bioagents is a prerequisite of biocontrol programme; this needs a regular and sufficient production of easily culturable factitious insect hosts for mass culturing of any bioagent. In India, rice moth, *C. cephalonica* (Pyralidae: Lepidoptera) popularly known as the flour moth or rice meal moth, is a major stored grain pest of many cereals and oilseeds. In India, rice meal moth is being utilized in various biocontrol research, developmental and extension units for mass production of number of natural enemies. It ranks first in the mass culturing of entomophagous insects due to its amenability to mass production, adaptability to varied rearing conditions and its positive influence on the progeny of the natural enemies. The important among them are-

- Egg parasitoids- *Trichogramma* spp.,
- Egg larval parasitoids – *Chelonus blackburnii* (Cameron),
- Larval parasitoids – *Bracon* spp., *Goniozus nephantidis* (Muesebeck),
- Insect predators – C*hrysoperla zastrowi sillemi* (Ebsen-Peterson)*, Mallanda boniensis* (Okamoto)*,*

Besides, some entomopathogenic nematodes such as *Steinernema feltiae* (Filipjev) are also reared on the larvae of *C. cephalonica*.

Due to the indiscriminate use of insecticides in pest management several problems *viz.,* development of insecticidal resistance in major pest of crops, resurgence and destruction of natural enemies besides ill effects of toxic residues on the environment have occurred. Taking into consideration several unintended repercussions of insecticides on agro-ecosystems, there was a revived serious concern about the environmental and health hazards. Hence, efforts are being made for an

ecologically sound and environmentally safe pest management system. Consequently, today in plant protection more emphasis is given in developing Integrated Pest Management (IPM) strategies. In IPM programme, greater reliance is given on biological control and non-chemical approaches. Several parasitoids and predators have been effectively used for the control of pest; their inundative releases as a means of pest management, subsequent to their mass production to a commercial scale, have become more popular.

Rearing technique of *Corcyra cephalonica* Stainton

The culture of *C. cephalonica* was obtained from naturally infested grain stored in a local warehouse. Broken sorghum grains were first sterilized at 121^0 C for 30 minute in a hot air oven. Larvae of *C. cephalonica* were reared on this heat sterilized broken sorghum grains (2.5 kg), groundnut powder (100 g), streptomycin (0.5 g) and powdered yeast (10 g) in a *C. cephalonica* rearing tray (45 cm x 30 cm x 10 cm). Normally, 0.25 cc (5000) eggs of rice moth were sprinkled in a *C. cephalonica* rearing tray and kept for development at 30±2^{0}C and 60±5% RH. *C. cephalonica* rearing tray containing full grown larvae was undisturbed for smooth pupation. The larvae pupate in silken cocoons. The moths started emerging from 30th day onwards and were collected daily either manually or with a device fitted with 0.5 HP vacuum pump. Collected moths were kept in *C. cephalonica* oviposition cages for egg deposition. It was fabricated from a 25-30 litre capacity plastic bucket with lid. The bottom portion was cut and covered with 40 size steel mesh and heat sealed. A window of 15 cm x 25 cm was cut on one side of the bucket and covered with the above mesh by heat sealing. A hole (1" diameter) was drilled in centre of the lid for fixing the tube. A window of 10 cm x 10 cm was cut on the lid and covered with the mesh by heat sealing. The wire mesh was nailed on each side of the window so that they were kept apart by the thickness of the lid. The fitting of the mesh in the lid was perfect enough to prevent entry of natural enemies like *Bracon hebetor* inside the boxes.

Handling of Adults

The adults are provided feed containing honey solution. The adult feed is prepared by mixing 50 ml honey with 50 ml water and 5 capsules of vitamin E (Evion). The feed is stored in refrigerator and used as and when required. Piece of cotton wool tied with a thread is soaked in the solution and inserted into the drum through the slot at the top. From a basin, moths can be collected up to 90 days after which the number of moths emerging dwindles down and keeping the basins is not economical for the producer.

HANDLING OF EGGS

The moths lay the eggs in large numbers loosely. The scales and broken limbs are also found in larger quantities along with the eggs. They cause potential hazard to the workers after years of working in *Corcyra* laboratory. To minimize the risk of scales freely floating in the air, the oviposition drums are placed on sheets of filter paper in enamel trays which trap effectively the scales. Sets of several oviposition drums are kept in ventilated place near an exhaust fan to enable the workers comfort. Daily morning the oviposition drums are lifted up and the

wire-mesh bottoms are cleaned gently with a shoe brush so that the eggs and remnants of scales and limbs settled on the mesh are collected along with those on the filter paper. The collections are cleaned by gently rolling the eggs on filter paper to another container. Then they are passed to sieves in series and finally clean eggs are collected. The eggs are quantified in measuring cylinders and used for building up the stocks and natural enemy production. About 100 pairs of adults produce 1.5 cc of eggs in 4 days laying period inside the oviposition drums.

Fig 2: Rearing cage of *C. cephalonica*

Fig 3: Collection of adults

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Wrky Transcription Factors: Key Regulators Of Plant Processes

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Transcription is the first step in gene expression and this process is regulated by transcription factors (TFs) which cause either activation or repression. More than 1500 TFs have been reported in Arabidopsis thaliana since 2000. The TFs contain a DNA binding domain (DBD) which specifically recognizes the target DNA sequence forming a transcriptional complex and thus regulate gene expression (Riechmann *et al.,* 2000, Guo *et al.,* 2005, Mitsuda and Ohme-Takagi, 2009). In Arabidopsis, TFs are categorized into many groups according to the conserved DBD domain, such as AP2/ERF (APETALA 2/ETHYLENE-RESPONSIVE ELEMENT BINDING FACTOR), B3 (the third basic domain in the maize gene VIVIPAROUS1), NAC (NO APICAL MERISTEM, ATAF1/2, CUP-SHAPED COTYLEDON2), SBP (SQUAMOSA-PROMOTER BINDING PROTEIN) and WRKY (TF containing a highly conserved WRKY domain) super families. One of the largest TF families is the WRKY super family, comprising plant specific TFs involved in many biotic and abiotic stress responses as well as plant growth and development (Rushton *et al.,* 2010). They show similarity with animal TFs, which contain the DNA-binding domain known as the GCM domain. TFs containing the GCM domain are classified together with WRKY TFs into the WRKY-GCM1 super family (Yamasaki *et al.,* 2013). To date, WRKYs are not restricted to higher plants such as Arabidopsis and other flowering plants, but are also found in, for example, ferns, slime mold and unicellular green algae, indicating the origin of WRKYs in primitive eukaryotes (Ulker and Somssich, 2004; Agarwal *et al.,* 2011; Yamasaki *et al.,* 2013).

On the basis of structural motifs, which can be either DNA-binding domain or functional domain of protein and fall into three categories (Buchanan *et al.,* 2000).

1. **Helix-turn-helix:** contains two alpha helical segments separated by inverting loop, which always bind to DNA as dimer. One helix of each monomer functions to keep the dimer together by interacting with its counterparts, whereas other helix create the scissors like structure which fits into the adjacent major grooves in the DNA.
2. **Basic leucine zipper:** this protein also creates a scissors like structure binds

to major grooves in the DNA. Each monomer contains alpha helix in which every seventh amino acid is leucine.

3. **Zinc finger:** protein does not form dimmers, rather than they create their own duplicated set of projections to insert into major grooves of DNA. The projections are created by coordinating the zinc ion with four amino acids (a combination of histidines and cysteines or just four cysteines).

WRKY Transcriptional factors:

The WRKY family is among the ten largest families of transcription factors in higher plants and is found throughout the green lineage (green algae and land plants). The family has expanded during the evolution of plants. This expansion is likely to be associated with the ongoing development of highly sophisticated defense mechanisms co-evolving in land plants together with their adapted pathogens. Significant advances regarding our understanding of WRKY proteins have occurred in the past ten years since the publication of the first review on WRKY transcription factors in 2000. However, more recently, research has focused on additional roles of WRKY factors in plant processes such as germination, senescence and responses to abiotic stresses such as drought and cold. Moreover, such studies are no longer restricted to model plants such as Arabidopsis (*Arabidopsis thaliana*) but are rapidly expanding to include in particular, crop species. In this review we will examine some of the recent advances in our knowledge in this rapidly moving area of plant research. This includes new information on the roles of WRKY transcription factors in plants, the mechanisms of WRKY protein function, auto regulation and cross-regulation in signaling involving WRKY transcription factors and the latest information about the evolution of WRKY genes based on recently sequenced plant genomes.

The WRKY domain and W box

The defining feature of WRKY transcription factors is their DNA binding domain. This is called the WRKY domain after the almost invariant WRKY amino acid sequence at the N-terminus. In a few WRKY proteins, the WRKY amino acid sequences have been replaced by WRRY, WSKY, WKRY, WVKY or WKKY. The WRKY domain is about 60 residues in length, and as well as containing the WRKY signature it also has an atypical zinc-finger structure at the C-terminus.

Regulation of WRKY transcription factors:

The notion of WRKY proteins involved in critical stress response obviously makes extensive regulation of the signaling pathway mandatory. In responses to both external and internal stimuli, WRKY protein binds to W- box containing promoters and triggers the expression of target stress-responsive genes. This triggering is often regulated by the WRKY protein itself or by separate WRKY TF's. Importance of autoregulation and cross regulation in maintain the homeostasis of WRKY protein expression in the cell. The tight regulation and the fine tuning of WRKY proteins during plant stress responses contribute to the establishment

of complex signaling webs. (Ligang *et al.,* 2011).

Functions of WRKY transcriptional factor:

1. Biotic stress:

The majority of reports concerning WRKY transcription factors have indicated that numerous members of the multigene family play roles in the transcriptional reprogramming associated with the plant immune response.

2. Abiotic stress:

WRKY transcription factors play pivotal roles in regulating many stress reactions in plants; however, unraveling their roles in abiotic stress responses has lagged behind that of biotic stresses. This may be a reflection of crosstalk and redundancy intrinsic to such responses and also a lack of suitable mutant lines. In one of the earliest studies, a WRKY gene isolated from a xerophytes evergreen C3 shrub, the creosote bush (*Larrea tridentata*), was shown to be an activator of abscisic acid (ABA) signaling. ABA mediates plant responses to abiotic stresses, and, hence is called a 'stress hormone'. In another transient expression study using aleurone cells, OsWRKY24 and OsWRKY45 were found to act as repressors of an ABA-inducible promoter, and OsWRKY72 and OsWRKY77 were shown to be activators of the same promoter. Some of the other earliest evidence of roles related to abiotic stress responses came from transcription profiling; however, recent functional analyses have provided more direct evidence. For example, in rice, heat shock inducible HSP101 promoter-driven over expression of OsWRKY11 led to enhanced heat and drought tolerance. Likewise, over expression of OsWRKY45 resulted in enhanced salt and drought tolerance, in addition to increased disease resistance.

3. Seed Development:

The role of WRKY genes in seed development is implicated in several gene expression studies. The WRKY transcription factor DGE1 of orchardgrass (Dactylis glomerata) is expressed during somatic embryogenesis. A Group a WRKY transcription factor in wild potato (Solanum chacoense), ScWRKY1, was found to be expressed strongly and transiently in fertilized ovules at the late torpedo stage. SUSIBA2 is expressed in the endosperm and regulates starch production.

4. Seed Dormancy and Germination:

In cereals, a-amylase enzymes are involved in hydrolysis of starch, an important step in germination and post germination growth of cereals. These genes are gibberellins (GA)-inducible and ABA-repressible and, hence, are ideal for GA and ABA crosstalk studies. In an early study of the WRKY family, two wild oat WRKY transcription factors, ABF1 and ABF2, were found to bind to W boxes in the promoters of the a-amylase gene a-Amy2. Several studies have demonstrated that rice and barley homologues of these two wild oat WRKY genes are ABA-inducible and GA-repressible in aleurone cells and embryos. *Via* bombardment-mediated transient expression in rice and barley, rice OsWRKY51 and OsWRKY71 were found to encode repressors of the rice RAmy1A a-amylase and the barley Amy32b a-amylase genes. By forming a

heterotetramer, OsWRKY51/71 antagonizes GAMYB, a well-documented transcriptional activator of GA signaling. Exogenous GA treatment destabilizes GFP:OsWRKY71 whereas the proteasome inhibitor MG132 blocks the degradation of this fusion protein. These lines of evidence suggest that OsWRKY51 and OsWRKY71 are key regulators mediating the crosstalk of GA and ABA in aleurone cells and embryos.

5. Senescence:

WRKY transcription factors are involved in the regulation of leaf senescence. Expression profiling in Arabidopsis revealed that WRKY transcription factors are the second largest family of transcription factors in the senescence transcriptome. The first evidence of a role in senescence came from studies of AtWRKY6. AtWRKY6 is strongly unregulated during senescence, and analysis of AtWRKY6 target genes identified the SENESCENCE-INDUCED RECEPTOR KINASE/FLG22-INDUCED RECEPTOR- LIKE KINASE (SIRK/FRK1). SIRK/FRK1 encodes a receptor-like protein kinase whose expression is strongly and specifically induced during leaf senescence. Other WRKY genes that regulate senescence include AtWRKY53, AtWRKY70 and OsWRKY23. Over expression or RNAi knockdown of AtWRKY53 led to senescence associated phenotypes, and a role for MEKK1 in this process has been determined. Knockout lines for AtWRKY70 showed that it acts as a negative regulator of senescence, and over expression of OsWRKY23 accelerated leaf senescence. This illustrates that members of the WRKY transcription factor family both positively and negatively regulate this process. Recently, epigenetic programming has also been implicated in the mechanism whereby AtWRKY53 regulates senescence.

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Extremophiles – Nature's Ultimate Survivors

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Extremophiles, an organism that are tolerant to environmental extremes and that have evolved to grow optimally under one or more of these extreme conditions, hence the suffix *phile*, meaning "one who loves." Extremophiles are organisms that have been discovered on earth that survive in environments that were once thought not to be able to sustain life. These extreme environments include intense heat, highly acidic environments, extreme pressure and extreme cold. Different organisms have developed varying ways of adapting to these environments, but most scientists agree that it is unlikely that life on Earth originated under such extremes.

Discovery of extremophiles

In 1966 Thomas Brock showed that microscopic organisms thrived in hot springs at Yellowstone National Park, USA. Since then extremophiles have been found all over the world, and their study is one of the rapidly expanding areas of biological science. Because of its variety of thermal features, New Zealand is one of the best places to study these organisms. Thermophiles were the first extremophiles to be discovered.

Characteristics of extremophiles

Extremophiles are organisms that live in extreme conditions of temperature, acidity, salinity, pressure, or toxin concentration. Most extremophiles are single-celled micro-organisms belonging to two domains of life – bacteria and archaea. These differ from fungi, plants, animals and other single-celled organisms because their genetic material is dispersed through the cell rather than being enclosed within a nucleus.

How extremophiles survive?

Unlike most organisms that require organic (carbon-containing) compounds for their energy or can carry out photosynthesis, some extremophiles can produce energy from inorganic compounds. The hot water found in geothermal areas is formed as the result of heating of groundwater by deep heat sources. Very hot water is highly corrosive. As it moves through fractures deep in the earth it can dissolve minerals or convert them to other minerals.

When the water reaches the surface, it forms hot spring fluids. These may contain high concentrations of dissolved chemicals such as chloride, sulfate, sodium, potassium, bicarbonate and silica. Also present are minor dissolved chemicals including calcium, iron, aluminum, arsenic, ammonia, hydrogen and hydrogen sulfide. Some of these provide the basic energy source and nutrients for a number of extremophile micro-organisms.

Classification and examples of extremophiles

Environmental Parameter	Type	Definition	Examples
Temperature	Hyperthermophile Thermophile Mesophile Psychrophile	Growth >80°C Growth 60-80°C 15-60°C <15°C	*Pyrolobusfumarii*, 113°C *Synechococcuslividis* *Homo Sapiens* *Psychrobacter*, Some Insects
Radiation			*Deinococcusradiodurans*
Pressure	Barophile Piezophile	Weight Loving Pressure Loving	Unknown For Microbe, 130 Mpa
Gravity	Hypergravity Hypogravity	>1g <1g	None Known None Known
Vacuum		Tolerates Vacuum (Space Devoid Of Matter)	Tardigrades, Insects, Microbes, Seeds
Desiccation	Xerophiles	Anhydrobiotic	*Artemiasalina*; Nematodes, Microbes, Fungi, Lichens
Salinity	Halophile	Salt Loving (2-5 M Nacl)	*Halobacteriacea,Dunaliellasalina*
pH	Alkaliphile Acidophile	Ph >9 Low Ph Loving	*Natronobacterium, Bacillus Firmus*of4, *Spirulina Spp.* (All Ph 10.5) *Cyanidium Caldarium*, *Ferroplasma Sp.* (Both Ph 0)
oxygen tension	Anaerobe Microaerophil Aerobe	Cannot Tolerate O_2 Tolerates Some O_2 Requires O_2	*Methanococcusjannaschii* *Clostridium* *Homo Sapiens*
chemical extremes	Gases Metals	Can Tolerate High Concentrations Of Metal (Metalotolerant	*Cyanidium Caldarium* (Pure Co_2) *Ferroplasmaacidarmanus*(Cu, As, Cd, Zn); *Ralstonia* Sp. Ch34 (Zn, Co, Cd, Hg, Pb)

TYPES OF EXTREMOPHILES

- **Psychrophiles** (extreme cold) — Organisms with the ability to survive at temperatures below –4°F. Many psychrophiles produce special proteins that act as antifreeze agents and prevent them from freezing solid. Some have evolved cell layers that resist hardening in extreme cold. An example of a psychrophile is *chryseobacterium greenlandensis*, which for the last 120,000 years has survived nearly two miles deep within the ice of a Greenland glacier.
- **Thermophiles** (extreme heat) — Organisms with the ability to survive at temperatures of 140°F or even higher.Similar to psychrophiles, thermophiles have developed special proteins that allow them to tolerate a broad range of temperatures, in some cases including temperatures above the boiling point of water. One example of a thermophile is *cyanidium*, an algae that lives in the hot water springs in Yellowstone National Park. Other thermophiles can be found in crater lakes, peat bogs and even in superheated hydrothermal vents on the deep ocean floor.
- **Radioresistant Microbes** (extreme radiation) — Organisms that can consistently survive doses of radiation that are 500 times greater than the lethal dose for humans. Radioresistant microbes often channel the energy from radioactivity to purposes such as producing food for themselves, and some have evolved aggressive DNA repair mechanisms to reverse any genetic damage caused by radiation. One example, *Deinococcus radiodurans*, is listed by the *Guinness Book of World Records* as "the world's toughest bacterium." Another type of radioactive fungi was found growing in the remains of the Chernobyl nuclear reactor following its "meltdown"in 1986.
- **Alkaliphiles** (extreme bases; high pH levels) — Organisms with the ability to survive and thrive in substances capable of neutralizing strong acids (environments with pH values ranging from 9 to 11). Alkaliphiles have evolved unique enzymes, specialized cytoplasm and efficient cell membranes to protect their cells from damage. One example are the colonies of the alkaliphile *Microcystis* that flourish in the extremely alkaline Mono Lake in California.
- **Acidophiles** (extreme acids; low pH levels) — Organisms that survive in highly acidic environments (where the pH value rarely rises about 2). Acidophiles are the opposites of alkaliphiles, thriving at the opposite end of the pH spectrum. *Ferroplasma acidiphilum* (found in mine drainage, waste treatment plants and acidic caves) is an acidophile that extracts energy from iron, essentially "eating" the metal and leaving behind rust.
- **Halophiles** (extreme saltiness) — Organisms that can survive in extremely salty environments (5 to 10 times saltier than ocean water). Halophiles coat themselves with a special protein layer that blocks excessive salt from entering its cells. *Dunaliella salina* is a halophile algae that lives in salt ponds and concentrates beta-carotene in its cell walls (resulting in an orange or pinkish color). Other halophiles have been found in Utah's Great Salt Lake, the Dead Sea between Israel and Jordan, and even growing on saltine crackers.

Tardigrada — an extreme of extreme life

Out of all of the extremophiles that have been discovered on Earth, most extreme survivors called tardigrada known as "Water bear". Tardigrada have been shown to survive:
Temperatures ranging from –200 °C (-328 F) to 151 °C (304 F)
Pressure ranging from near vacuum (like that found in outer space) to 1,200 times atmospheric pressure
Dehydration and lack of water (one documented specimen survived in a dehydrate state for nearly ten years)
Massive doses of radiation, as high as 5,000 Gy (Gray units; 5–10 Gy are typically lethal to humans)

- **Xerophiles** (extreme dryness; lack of water) — Organisms that can grow and reproduce in conditions with very little water available. Xerophiles have evolved means to store and conserve any water they encounter, so it is available when needed (even if there is no water left in the surrounding environment). *Wallemia sebi* is a xerophile mold that grows in dried fruit, salted meats and even the evaporation beds where sea salt is produced. Mold growth on bread is an example of food spoilage caused by xerophilic organisms.
- **Barophiles** (extreme pressure) — Organisms that live in highly pressurized environments, such as the bottom of the ocean. Barophiles have evolved a waxy cell layer which protects against both crushing pressures and frigid temperatures. Just to stay alive, the barophile *Halomonas salaria* requires pressure 1,000 times that found at Earth's surface. Most barophiles are found on the ocean floor, where pressures are at least 400 times higher than Earth's surface.
- **Endoliths** (extreme rockiness) — Organisms with the ability to survive within solid rock, or deep within the Earth's crust. Endoliths can survive for hundreds of years by feeding on trace amounts of iron, potassium and sulfur found in the rocks they inhabit. Some endoliths have been found as deep as two miles in the Earth's crust, while others are found in desert rocks and on mountain slopes. Many scientists think that endoliths are the type of life most likely to be found on Mars (either living

there today, or having existed there some time in the past).

IMPORTANCE OF EXTREMOPHILES

The diversity of extremophiles are found in extreme environments. Extremophiles have enzymes known as extremozymes which are important in many application. The products likewise different enzymes are important in industry and clinical diagnosis. The biotechnology of extremophiles gives various approaches for molecular biological techniques. The unparallel enzymes "extremoenzymes" used by the extremophiles to carry out their biochemical processes in harsh environments are useful in biotechnological processes. The property of capability of surviving under hard conditions by the enzymes, such as ability to function at very high pressure and temperature are major tools in biotechnolological research. Popular example is the so called taq polymerase enzyme isolated from the extremophile Thermus aquaticus is an essential part in PCR (polymerase chain reaction) technique that has brought radical change in biotechnology. And the most extraordinary microbe Deinococcus radiodurans is able to withstand high levels of lethal ionizing radiation.

Extremophiles have made an important contribution to biology & challenging new ideas about origin & evolution of life on earth have been generated. The study of extremophiles and extremozymes provides information about protein folding , stability , structure and function. The extrmozymes have great economic potential in many industrial process. Microbial communities continue to be discovered in environments once thought to be too hostile to support any form of life. The viruses of some of these extremophiles, which also have significant interest for controlling microbial community structure or as sources of extremozymes. Extremophiles are also of research importance in the field of astrobiology.

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The Impact Of BCG Vaccination In Cattle

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Bacille Calmette Guerin (BCG) is the current vaccine for tuberculosis. It was first used in 1921. BCG is the only vaccine available today for protection against tuberculosis. It is most effective in protecting animal from the disease. The *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) vaccine, an attenuated strain of *M. bovis*, was developed for control of human tuberculosis more than 90 years ago and is still the only tuberculosis vaccine available. The timing of BCG vaccination could be critical. It may be preferable to vaccinate infants as neonates prior to any exposure to environmental mycobacteria, but at this age the type and degree of immune response may not be optimal.

HISTORY OF THE VACCINE

Bacille Calmette Guerin (BCG) containes a live attenuated (weakened) strain of Mycobacterium bovis. It was originally isolated from a cow with tuberculosis by Calmette and Guren who worked in Paris at the Institute Pasteur. This strain was carefully subcultured every three weeks for many years. After about thirteen years the strain was seen to be less virulent for animals such as cows and guinea pigs. During these thirteen years many undefined genetic changes occurred to change the original stain of M. bovis. This altered organism was called BCG. In addition to the loss of virulence, other changes to BCG were noted. These included a pronounced change in the appearance of colonies grown in the laboratory. Colonies of M. bovis have a rough granular appearance whereas colonies of BCG are moist and smooth.

STRAINS OF BCG

BCG was first used as a vaccine to protect humans against tuberculosis in 1921. At first, cultures of BCG were maintained in Paris. Later, it was subcultured and distributed to several laboratories throughout the world where the vaccine strain called BCG continued to be maintained by continuous subculture. After many years it became clear that the various strains maintained in different laboratories were no longer identical to each other. Indeed, it was likely that all the various strains maintained by continuous subculture continued to undergo undefined genetic changes. Indeed, the "original" strain of BCG maintained at in Paris had continued to change during the subcultures needed to maintain the viability of the culture. To limit these

continuing changes the procedures needed to maintain the strain were modified. Today, the organism is maintained in several laboratories using a "seed lot" production technique to limit further genetic variation using freeze-dried (also called lyophilized) cells so that each batch starts with the same cells.

SAFETY

After extensive tests in animals, BCG was first used as a vaccine in 1921. It was given orally to infants. Since this time the vaccine has been widely used. BCG is widely used and the safety of this vaccine has not been a serious issue until recently. There is a concern that use of the vaccine in persons who are immune compromised may result is an infection caused by the BCG itself. Also, even among immune competent persons, local reactions, including ulceration at the site of vaccination may result in shedding of live organisms which could infect others who may be immune compromised.

The early use of BCG was marked by a tragic accident. In Lubeck more than 25% of the approximately 250 infants who received a batch of the vaccine developed tuberculosis. It was later recognized that this batch was accidentally contaminated with a virulent strain of M. tuberculosis.

BCG production and substrains

The BCG vaccines that are currently in use are produced at several sites throughout the world. These vaccines are not identical. To what extent they differ in efficacy and safety in animals is not clear at present. Some differences in molecular and genetic characteristics are known. What is not known is if the "BCG" from one manufacturer is "better" than one produced at another site. Each BCG is now known by the location where it is produced. For example, we have BCG (Paris), BCG (Copenhagen), BCG (Tice) and BCG (Montreal) among others.

Roles for innate immune effectors in the immune response induced by BCG vaccination or M. bovis infection

An important difference between neonatal calves and older animals that could be relevant to vaccination and immune response induction is the relatively high circulating numbers of innate cells in young calves. In particular, increased circulating numbers of gamma-delta TCR-expressing T cells expressing the workshop cluster-1 (WC1) receptor can constitute up to 60% of the PBMC population in calves and numbers of natural killer (NK) cells were also highest in very young calves We therefore hypothesised that the enhanced protection observed following BCG vaccination of neonates is associated with increased numbers of WC1 T cells and NK cells, and that appropriate immune response induction is orchestrated by complex interactions between these innate lymphocytes and dendritic cells (DC). This could then facilitate optimal activation of CD4+ and CD8+ T lymphocytes for protective immunity. Dendritic cells are the only capable of stimulating naive T cells and are pivotal in the induction of immune responses. Murine studies have elucidated that the effective control of mycobacterial infection is reliant upon transport of antigen by migratory DC to draining lymph nodes to effectively prime CD4+ T cells and subsequently polarize Th1 biased immune

responses. In order to become fully active, DC require additional signals from the innate immune system, in addition to pathogen derived signals. In particular, the ability of DC to secrete biologically active IL-12 has been shown to be dependent on the presence of IFNγ which may be secreted in high concentrations by innate immune cells such as NK cells. Reciprocal interactions may occur between DC and other innate immune cells whereby activation of DC enhances their capacity to stimulate T lymphocyte responses, and increased secretion of of IFNγ by innate effectors contributes to Th1 polarisation. These interactions are likely to significantly affect anti-mycobacterial immunity.

T-cell responses to mycobacteria

Inoculation of mice, humans and cattle with mycobacteria induces antigen specific CD4+, CD8+ and gd T-cell responses .Inoculation of cattle with M. bovis induced changes in the peripheral blood lymphocyte (PBL) composition, with an early increase in the percentage of gd-WC1+ T-cells ,indicating that this T-cell subtype may play a role in the early control of mycobacteria growth. Depletion of WC1+ cells at the time of M. bovis infection resulted in higher levels of IL-4 production by PBL stimulated in vitro with mycobacterial antigens compared to control animals. This change was accompanied by a decrease in the production of IgG to mycobacterial antigens .although no changes in pathology were detected at post-mortem. CD4+ and CD8+ T-cells are essential for immunity to mycobacteria, as individuals deficient in either T-cell subtype are more susceptible to infection [54–58]. Mice deficient in CD4+ T-cells are more susceptible to mycobacteria than mice deficient in CD8+ T-cell, which in turn are more susceptible than their wild type counterparts [57,58]. In humans, in particular HIV+ patients, individuals with low CD4+ T-cell counts have a greater susceptibility to mycobacterial infections than individuals with normal CD4+ T-cell counts. In vivo experiments to determine the role of CD4+ T-cells in immunity to mycobacteria in cattle have not been carried out. However, it has been shown that peripheral blood CD4+ cells are the main producers of IFNg following stimulation in vitro with mycobacterial antigens and therefore are thought to play an important role in immunity against mycobacteria .In vivo experiments to determine the role of CD8+ T-cells in immunity to mycobacteria in cattle have indicated that CD8+ T-cells indeed play a role in the immune response to mycobacteria but may also play a role in the immunopathogenicity of bTB .Both T-cell subsets contribute to immunity to mycobacteria by production of IFNg and activation or killing of infected cells .However, killing the infected cells can result in either the release of intracellular bacteria or the killing of both the infected cell and the bacteria. The release of mycobacteria from infected APC can in turn result in spread of infection or uptake of released mycobacteria by activated MO able to kill the pathogen. Thus, the most efficient way of clearing the infection would be the killing of intracellular mycobacteria at the same time as the death

of the infected host cell. Once the infection has been established, if it is controlled but not all the mycobacteria have been killed, their subsequent growth is governed by CD4+ and CD8+ T-cells. Both T-cell types have been shown necessary for the maintenance of a latent state of infection .Thus, CD4+ and CD8+ T-cells contribute to the formation of the TB granuloma and to the arrest of mycobacterial growth mainly by the expression of a Th1 type response. This response may be sufficient to contain the growth of mycobacteria and to account for the absence of disease. Alternatively, if the infection is not contained the granuloma will evolve. Bovine TB granulomas have been classified into four stages according to cellular composition and degree of necrosis. Stage 1 granulomas exhibit no necrosis and are composed mainly by epithelioid MO, few giant cells and interspaced lymphocytes. Stage 4 granulomas exhibit extensive necrosis and are composed of epithelioid MO surrounding a necrotic nucleus and WC1+ T- and B-cells at the margins of the granuloma. Typical TB lesions are calcified granulomas and the reasons for the development and calcification of the TB granuloma are not clear. It has been shown that development of granulomas occurs as early as 5 weeks after experimental infection of cattle and is associated with development of antigen specific peripheral blood immune responses .It has been shown that interplay of Th1 and Th2 cytokines occurs in the mycobacteria granuloma .Thus, GM-CSF, IFNg, TNFa, TGFb, IL-1 IL-4, IL-10, and IL-12 have been detected in tissues containing TB lesions TNFa and IFNg are essential cytokines for resistance to mycobacteria, which are produced by components of both the innate and adaptive immune response However, it has been shown in cattle infected experimentally with M. bovis that the level of antigen specific production of IFNg by peripheral blood cell in vitro is associated with the degree of pathology. Thus, while necessary for protection, IFNg may also play a role in the immunopathogenesis of bTB. A better understanding of the role of the immune response in the formation and development of TB granulomas is necessary for the development of vaccines that will trigger immune protective, rather than immune pathogenic responses. Overall, understanding the complex interplay between APC, innate and adaptive immune components and mycobacteria will allow more informed vaccine design, facilitate the development of accurate tests allowing prediction of vaccine efficacy and assist in development of accurate diagnostic tools for the control of TB.

The future of TB vaccines

Early immune events are likely to be pivotal in determining the outcome of infectious challenge, and vaccination must stimulate appropriate immune responses.It is now well established that the major host cells for mycobacteria: MO and DC,display divergent responses to infection with mycobacteria. This response is likely dependent upon unique expression patterns of PRR by MO and DC. Early responses of DC dictate the nature of the subsequent adaptive immune response and

as such, vaccines targeting specific responses of DC hold significant promise. As it is likely that any future vaccination strategy for TB will be based on BCG either alone or in a prime-boost protocol, it would be of interest to explore whether BCG priming can be specifically targeted to DC, by means of coating bacteria with antibodies to molecules expressed preferentially by DC at different anatomical sites. This would avoid the use of DC-SIGN by BCG and preferentially induce protective immune mechanisms. The capacity of targeted DC to stimulate appropriate innate and early adaptive immune responses leading to protective immunity would be central to the success of this type of vaccination strategy. In prime boost strategies, the booster component of the strategy would most likely be a recombinant antigen-expressing vector, such as MVA or adenovirus or non-replicating antigens mixed with adjuvant and would seek to amplify, rather than modify, the primary immune response and would target MO whilst avoiding the activation of B-cells. Humoral immune responses have been associated with development of pathology in TB. By contrast, cell mediated immunity mediated by both CD4+ and CD8+ T-cells responses have been associated with protective immune responses against mycobacteria. In a successful prime boost vaccination strategy, the priming component would elicit CD4+ T-cell responses, while the booster component would boost CD4+ T-cells and elicit CD8+ T-cells. As our understanding of the requirements for protective immunity increases so does the potential for targeted vaccination. Successful vaccination against TB is now, more than ever, a realistic goal. Translation of experimental findings to the field is a major challenge

CONCLUSIONS

Significant progress has been made in the development of TB vaccines for cattle. Most significantly, in parallel with developments in the human TB vaccinedevelopment field, vaccine strategies are now being considered for supplementing rather than replacing BCG vaccination. In particular, subunit vaccines based on DNA or proteins in adjuvant used in combination with BCG have resulted in better protection against experimental challenge with M. bovis than BCG vaccination on its own. BCG vaccination of neonates has also proved to be highly protective. Moreover, reagents that allow discrimination between vaccinated and infected animals have been developed, and comparative genomics has been found to be a useful method to identify target antigens. Finally, correlates of disease severity have been identified that could help predict the success or failure of vaccination with some confidence thus potentially shortening experimental protocols.

Canine Vaginal Cytology

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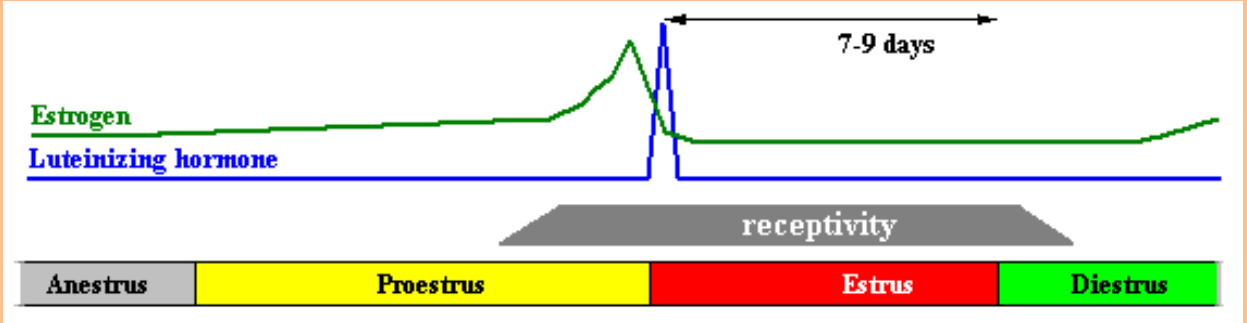
Stages of the canine estrous cycle can be defined by sexual behavior, physical signs (vulvar swelling, vaginal bleeding) or by vaginal cytology. The period of receptivity to a male varies considerably among bitches; some bitches are receptive well before and after the period of potential fertility. Similarly, signs such as "proestrus bleeding" are often unreliable indicators; some bitches bleed very little and other show bleeding through estrus and into diestrus. Since cytologic changes reflect the underlying endocrine events of the cycle, they are almost always a better predictor of the "fertile time" and gestation length than are behavioral or physical signs.

Cytologic changes through the canine estrous cycle reflect changes in blood concentrations of estrogen. As depicted below, the estrogen levels rise prior to and during proestrus and fall in conjunction with the preovulatory surge of luteinizing hormone. Rising levels of estrogen induce the "cornification" that is characteristic of smears examined during estrus. Ovulation occurs two days after the LH surge. The sections below describe the cytologic picture typical of different stages of the canine estrous cycle. Examination of a single smear can sometimes provide useful information, but can also be quite misleading. For example, it is often difficult to differentiate proestrus and diestrus from an isolated smear. It is therefore highly recommended that multiple smears be evaluated.

TECHNIQUE:

- Moisten a cotton swab with 1 to 2 drops sterile saline. Open the vulvar lips, pull the vulva dorasslly, insert the swab dorsally and posterior, then up and over the pelvic brim and into anterior vagina. If you do not pass the swab far enough, you will get vestibular cells and result in false

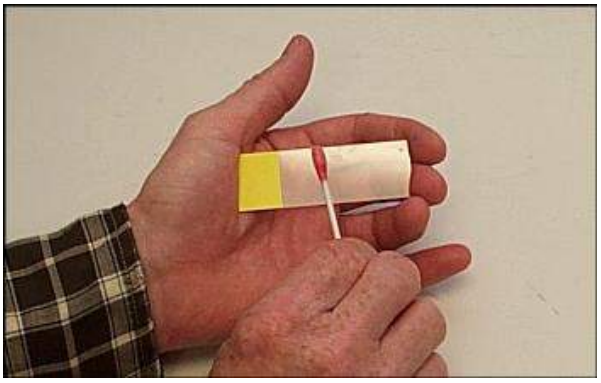


cornification. If you pass the swab too ventral, you may enter the bladder and get a falsely non-cornified smear.

- Roll the swab firmly onto a slide.
- Stain the slide using DifQuik stain, 10 dips in A, 15 dips in B, and 20 dips in C. You may also use new m**e**thylene blue stain.
- Read the slide under low power first to establish the trend of cellularity and cell types. Move to a higher power to establish the cell types. View several fields to get an overall visual idea of the percentage of cornified cells.

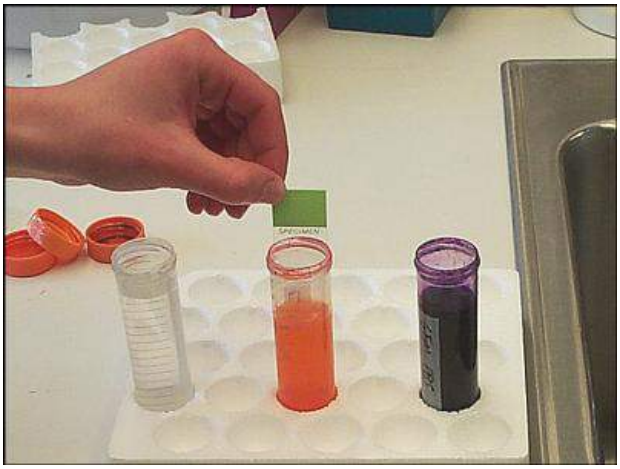
Preparing and Staining the Smear

Prepare the smear immediately after withdrawal of the swab by *rolling* (not sliding or rubbing) the cotton tip along the length of a glass microscope slide. Generally, two parallel tracks can be rolled on a single slide.



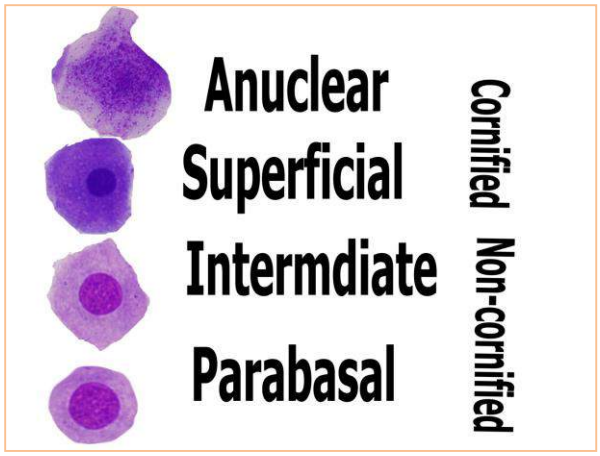
As soon as the smear is prepared, dip it 5 to 10 times in a container of methanol, or fix by use of a spray fixative. It is best to fix expeditiously, but allowing the slide to dry fully and remain unfixed for up to a few hours is generally not a problem. After fixation, the slide can be stored for prolonged periods of time, although typically, it is stained without delay.

Diff-Quik is a very convenient stain for use with vaginal smears. It consists of two solutions: an eosinophilic (red) solution and a basophilic (blue) stain. It is best to have a small container of each component and replace the stains with some regularity, as they will become contaminated with vaginal cells after staining a number of slides. To stain the smear, dip the slide in and out of the red stain 5 to 10 times, the in and out of the blue stain 5 to 10 times.



There is no need to rinse the slide between red and blue, but do not dip first into the blue, then into the red, or the red will rapidly become blue! If the staining appears too light or too dark, adjust the number of dips accordingly. A number of other stains can be substituted for Diff-Quik.

After dipping in the blue stain, rinse the slide in tap water and it's ready to examine. Examine while still wet or dry the slide and apply a coverslip.



Non-cornified

- Parabasal cells have a large stippled nucleus and a rounded cytoplasm The nucleus is large compared to the cytoplasm.
- Intermediate cells have a have a stippled nucleus and more cytoplasm than parabasal cells. The cytoplasm may even become angular.

Cornified

- Superficial cells have a pyknotic nucleus and angular cytoplasm. There is no stipling in the nucleus.
- Anuclear cells have no visible nucleus and angular cytoplasm.

Changes during the estrous cycle

The vaginal epithelium is responsive to sex steroids, particularly estrogen, and undergoes predictable changes through the cycle in response to changes in blood concentrations of ovarian hormones. Rising levels of estrogen cause the vaginal epithelium to become "*cornified*" - the surface cells become large and flattened, with small or absent nuclei.

- When no estrogen is present (anestrus and diestrus, the vaginal wall is very thin and is comprised of noncornified cells. In anestrus there will be very few cells and what you see will be debris and non-cornified cells.
- When estrogen rises during proestrus, the vaginal epithelium becomes hyperplastic and more cornified. During proestrus the percent of cornified cells increases by about 10%/day until you see about 100% cornification during estrus. You may also see RBCs during proestrus.
- During estrus the vaginal epithelium is very thick. You will see almost 100% cornification. The smear will look the same from the first day of estrus of the last day of estrus, You cannot tell which day of estrus the bitch is in based on vaginal cytology. There may however, be some sheeting of cells during the last 1-2 days of estrus. The vaginal wall is so thick during estrus that PMNs do not cross the epithelium. This makes the background look very 'clean'.
- On the first day of diestrus the cells in the swab abruptly change to around 50% non-cornified. This day that the smear changes from 100% cornified cells to 50% non-cornified cells is denoted as the first day of cytologic diestrus. You may see an influx of PMNs at this time to help clean up all the cellular debris.

Patterns of' Vaginal Stmiears During Cyclic Change

Under estrogen influence, during proestrus and early estrus, the cells of the vaginal epithelium begin to proliferate, differentiate, and exfoliate. As a result, the

number of cell layers increase and the predominant cell type on the surface changes rapidly from basal and parabasal to intermediate, and then to a superficial cell type. It is now possible to compile a superficial cell index (SCI) and a karvopyknotic cell index (KPI). A total number of 100 or 200 cells are counted. As proestrus progresses the SCI will rapidly approach 100% and remains high throughout estrus. The KPI increases slowly and reaches 80% by estrus and then increases near 100% at ovulation. Both the SCI and KPI will fall sharply after ovulation at the onset of metestrus. The beginning of metestrus can also be determined by the subtle appearance of neutrophils, parabasal and small intermediate cells in the vaginal smear.

SCI = Number of cells from superficial epithelial layers x 100
Total number of epithelial cells

KPI = Number of anuclear cells or cells with pyknotic nuclei x 100
Total number of superficial cells

Anestrus

Very few cells are usually encountered during this phase. The cells present are usually of the intermediate and parabasal type with a moderate number of neutrophils present or absent.

Proestrus and Estrus

During early proestrus the cells become more differentiated and all cell types encountered are superficial, intermediate and parabasal epithelial cells. Erythrocytes are also present and neutrophils may be seen during the first few days. By midproestrus the neutrophils are absent as are the parabasal and intermediate cells. In late proestrus virtually only superficial cells are present in the smear although red blood cells may still be seen. Late proestrus and early estrus are difficult to distinguish with vaginal smears. The vaginal smears during late proestrus and early estrus become clear due to a disappearance of debris with only pyknotic and anuclear superficial cells being present. Mid estrus is characterized by the presence of superficial cells, most of which are anuclear and the background of the smear is clean and clear. Erythrocytes may or may not be present during estrus.

Met estrus

There are numerous cells which are of the intermediate and parabasal variety. A few superficial cells may be seen during metestrus. A characteristic phenomenon is the very high number of neutrophils in this phase which reappear as soon as metestrus starts. If parabasal or intermediate cells are infiltrated by white blood cells they are referred to as metestrus cells. Foam cells, parabasal cells that contain cytoplasmic vacules, may also be seen during this phase of
the cylce. There is no entity in the clinical evaluation of the reproductive cycle as important as vaginal cytology. Many infertility problems can be corrected by simply interpreting vaginal cytology. The breeding cycle of each bitch may vary and many breeders and veterinarians may be misled by these variations. It has been recommended for cytological examination, that cells be obtained from the vagina rather than the vulva. However, others feel that it is unnecessary to collect material from the anterior vagina in the bitch.

Applications of Major Enzymes in Food Industry

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Enzymes are proteins that act as catalysts in all living organisms - microorganisms, plants, animals, and humans. Catalysts are compounds that increase the rate of chemical reactions in biological systems. Very small quantities of enzymes can increase the rate of reactions up to ten million times. Enzymes operate within a narrow set of conditions, such as temperature and pH (acidity), and are subject to inhibition by various means. Enzymes are classified by the type of reaction they catalyse and the substance (called a substrate) they act upon. It is customary to attach the suffix "ase" to the name of the principle substrate upon which the enzyme acts. For example, lactose is acted upon by lactase, proteins by proteases, and lipids by lipases. Additionally, many long-used enzymes have common names, such as papain, from papaya, which is used to tenderize meat.

History of Enzyme Use in Food Production

Enzymes extracted from edible plants and the tissues of food animals, as well as those produced by microorganisms (bacteria, yeasts, and fungi), have been used for centuries in food manufacturing. Rennet is an example of a natural enzyme mixture from the stomach of calves or other domestic animals that has been used in cheese making for centuries. Rennet contains a protease enzyme that coagulates milk, causing it to separate into solids (curds) and liquids (whey). Alternatively, for centuries enzymes produced by yeast have been used to ferment grape juice in order to make wine.

Modern Production of Food Enzymes

In the twentieth century, enzymes began to be isolated from living cells, which led to their large-scale commercial production and wider application in the food industry. Today, microorganisms are the most important source of commercial enzymes. Although microorganisms do not contain the same enzymes as plants or animals, a microorganism can usually be found that produces a related enzyme that will catalyse the desired reaction. Enzyme manufacturers have optimized microorganisms for the production of enzymes through natural selection and classical breeding techniques.

Direct genetic modification (biotechnology) encompasses the most precise methods for optimizing microorganisms for the production of enzymes. These methods are used to obtain high-yielding production organisms. Biotechnology also provides the tools to have a genetic sequence from a plant, animal, or a microorganism, from which commercial scale enzyme production is not adequate, to be transferred to a microorganism that has a safe history of enzyme production for food use.

Although the production organism is genetically modified the enzyme it produces is not. Enzymes produced through biotechnology are identical to those found in nature. Additionally, enzymes produced by microorganisms are extracted and purified before they are used in food manufacturing. Genetically modified microorganisms are useful from a commercial standpoint but would not survive in nature.

Major enzyme used in food industry

In food industry, enzyme has been used to produce and to increase the quality and the diversity of food. Some examples of products that use enzyme are cheese, yoghurt, bread syrup etc. Ancient traditional arts such as brewing, cheese making, meat tenderization with papaya leaves and condiment preparation (eg. soy sauce and fish sauce) rely on proteolysis, albeit the methods were developed prior to our knowledge of enzymes. Early food processes involving proteolysis were normally the inadvertent consequence of endogenous or microbial enzyme activity in the foodstuff. Some major applications by types of enzymes are:

1. Amylases: They can be derived from bacteria and fung|, They play a major role In the food and beverages (baking)) browing, starch, sugar industries. Amylase is used to hydrolyze amilum in to a product that is water soluble and has low molecular weight; glucose. This

enzyme is used, extensively in drink Industry for example the production of *High Fructose Syrup* (HFS) or in textile industry.' Amylases can be made from various microorganisms especially from Bacillus, Pseudomonas and Clostridium family. Potential bacteria that are recently used to produce amylases in industrial scale are *Bacillus licheniformis* and *B. stearothermophillus*. Alpha amylases have significant effects on baked goods. If the content is low, this leads to low dextrin production and poor gas production. This in turn results in inferior quality bread with reduced size and poor crust colour. To compensate for the deficiencies of the grain, It is necessary to add either sugar or alpha amylase.

2. Catalases: Catalase in an enzyme that can be produced from bovine liven or microbial sources. It is used to change hydrogen peroxide to become water and oxygen molecules. Thin enzyme can be used in a limited amount in cheese production. Catalase to the enzyme that breaks down hydrogen peroxide to water and molecular oxygen. Catalase effectively removes the residual hydrogen paroxide, ensuring that the fabric to peroxide-free and mainly used in food industry and also in egg processing with other enzymes. Catalans to a common enzyme found in nearly all living organisms which are exposed to oxygen, where it functional to catalyze the decomposition of hydrogen peroxide to water and oxygen. Glucose oxidase and catalase are often used together in selected foods for preservation, Superoxide dismutase to an antioxidant for foods and generates H_2O_2, but to more affective when catalase to present.

3. Lactases: Lactose, the sugar found in milk and whey, and its corresponding hydrolase, lactase or b-galactosidase has been extensively researched during the peat decade. This because of the enzyme immobilization technique which has given new and interesting possibilities for the utilization of this sugar. Because of intestinal enzyme insufficiency, some individuals, and even a population, show lactose intolerance and difficulty in consuming milk and dairy products. Hence, low-lactose or lactose-free food aid programme is essential for lactose-intolerant people to prevent severe tissue dehydration, diarrhoea, and, at times, even death. Another advantage of lactase treated milk is the increased sweetness of the resultant milk, thereby avoiding the requirement for addition of sugars in the manufacture of flavoured milk drinks. Manufacturers of ice cream, yoghurt and frozen desserts use lactase to improve scoop and creaminess. sweetness, and digestibility, and to reduce sandiness due to crystallization of lactose in concentrated preparations. Cheap source of lactose for the production of lactic add by fermentation. The whey permeate, which is a by-product in the i of whey protein concentrates, by ultra could be fermented efficiently by *Lactobacillus bulguricus*. Lactose can be obtained from various sources like plants, animal organs, bacteria, yeasts (Intracellular enzyme), or molds.

4. Lipases: A lipase to water -soluble enzyme that catalyzes the hydrolysis of ester bonds in water-insoluble, lipid substrates. Lipases (triacylglycerol acylhydrolases) are produced by microorganism in Individual or together with esterase. Micro-organisms that

produce lipases are *Pseudomonas aeruginosa*, *Serratia marcescens*, *Staphylococococcus aureus* and *Bacillus subtilis*. Lipase is used as bio-catalyst to produce free fatty acid, glycerol and various esters, part of glycerides and fat that is modified or esterified from cheap substrate i.e. palm oil. Those products are extensively used in pharmacy, chemical and food industry. Various animal or microbial lipases gave pronounced cheese flavour, low bitterness and strong rancidity, while lipases in combination with proteinases and/or peptidases give good cheese flavour with low levels of bitterness.

5. Proteases: The proteolytic system of lactic acid bacteria is essential for their growth in milk, and contributes significantly to flavour development in fermented milk products. The proteolytic system is composed of proteinases which cleave the peptides to small peptides and amino acids; and transport system responsible for cellular uptake of small peptides and amino acids. Ladle acid bacteria have a complex proteolytic system capable of converting milk casein to the free amino acids and peptides necessary for their growth. These proteinases include extracellular proteinases, endopeptidases, aminopeptidases, tripeptidases and proline-specific peptidase, which are all serine proteases. Apart from lactic streptococcal proteinases, several other proteinases from nonlactostreptococcal origin have been reported. There are also serine type of proteinases, e.g. proteinases from *Lactobacillus acidophillus*, *L. plantarum*, *L. diebrueckii* sp. *bulgaricus*, *L. lactis* and *L. helveticus*. Aminopeptidases are Important for the development of flavour in fermented milk products.

6. Rennet: The use of rennet in cheese manufacture was among the earliest applications of exogenous enzymes in food processing, dating back to approximately 6000 BC. The use of rennet, as an exogenous enzyme, in cheese manufacture is perhaps the largest single application of enzymes in food processing. In recent years, proteinases have found additional applications in dairy technology, for example in accelerations of cheese ripening, modification of functional properties and preparation of dietic products. Animal rennet (bovine chymosin) is conventionally used as a milk-clotting agent in dairy industry for manufacture of quality cheeses with good flavour and texture Marty microorganisms are known to produce rennet like proteinases which can substitute the calf rennet. Microorganisms like *Rhizomucor pusillus*, *R. miehei*, *Endothia parasitica*, *Aspergillus oryzae* and *Irpex lactis* are used extensively for rennet production in cheese manufacturing.

Table 1: Some uses of enzymes in food production

Market	Enzyme	Purpose / function
Dairy	Rennet (protease)	Coagulant in cheese production
	Lactase	Hydrolysis of lactose to give lactose-free milk products
	Protease	Hydrolysis of whey proteins
	Catalases	Removal of hydrogen peroxide

Brewing	Cellulases, beta-glucanases,alpha amylases, proteases, maltogenic amylases	For liquefaction, clarification and to supplement malt enzymes
Alcohol production	Amyloglucosidase	Conversion of starch to sugar
Baking	Alpha-amylases	Breakdown of starch, maltose production
	Amyloglycosidases	Saccharification
	Maltogen amylase (Novamyl)	Delays process by which bread becomes stale
	Protease	Breakdown of proteins
	Pentosanase	Breakdown of pentosan, leading to reduced gluten production
	Glucose oxidase	Stability of dough
Wine and fruit juice	Pectinase	Increase of yield and juice clarification
	Glucose oxidase	Oxygen removal
	Beta-glucanases	
Wine and fruit juice	Pectinase	Increase of yield and juice clarification
	Glucose oxidase	Oxygen removal
	Beta-glucanases	
Meat	Protease	Meat tenderising
	Papain	
Protein	Proteases, trypsin, aminopeptidases	Breakdown of various components
Starch	Alpha amylase, glucoamylases, hemicellulases, maltogenic amylases, glucose isomerases	Modification and conversion (eg to dextrose or high fructose syrups)
		dextranases, beta-glucanases
Inulin	Inulinases	Production of fructose syrups

Table 2: Marketed enzymes produced using gene technology

Principal enzyme activity	Application
Alpha-acetolactate decarboxylase	Brewing
Alpha-amylase	Baking, brewing, distilling, starch
Catalase	Mayonnaise
Chymosin	Cheese
Beta-glucanase	Brewing
Alpha-glucanotransferase	Starch
Glucose isomerase	Starch
Glucose oxidase	Baking, egg mayonnaise
Hemicellulase	Baking
Lipase	Fats, oils
Maltogenic amylase	Baking, starch
Microbial rennet	Dairy
Phytase	Starch
Protease	Baking, brewing, diary, distilling, fish, meat, starch, vegetable
Pullulanase	Brewing, starch
Xylanase	Baking, starch

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Thermal Processing of Milk: A Greater Way to Make

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THERMAL PROCESSING:

The term thermal processing applies to a range of heat treatments used for food processing. In general, the point of thermal processing is to kill pathogens and inactivate enzymes that cause negative changes to the food during storage.The most common type of thermal processing is the kind that happens in the kitchen at meal time. Even the most domestically challenged among us have heated something in the microwave and have therefore "thermally processed" something.

OBJECTIVE OF THERMAL PROCESSING:

The concept of thermal processing, which primarily involves in-container sterilization of foodstuff has come a long way since **Bigelow** and **Ball** developed in 1920, the first scientific basis for calculating the minimum safe sterilization process. In all its forms of application, thermal processing persists as the most widely used method of preserving and extending the useful shelf life of foods. The concept of in-container sterilization(canning)involves the application of high-temperature thermal treatment for a sufficiently long time to destroy microorganisms of public health and spoilage concerns. The hermetic seal maintains an environment in the container that prevents the growth of other microorganisms of higher resistance and most importantly prevents recontamination and pathogens from producing toxins during storage. The demand for processed foods goes beyond the fundamental requirements of safety and shelf-stability. More emphasis is being placed on informatively labeled, high-quality, and value-added foods with convenient end use. Improvements in quality and safety of processed foods have been achieved through regulatory requirements on manufacturers, and national or international legislature that recommend and/or enforce performance standards or methods for achieving safety and quality assurance. Equally important is the fact that the need for affordable, yet, high value-added products has been driven by the consumer. Conventional canning operations have the tendency to induce permanent changes to the nutritional and sensory attributes of foods. Therefore, recent developments in food processing operations have aimed at technologies that have the potential to substantially reduce damage to nutrients and sensory components by way of

reduced heating times and optimized heating temperatures.

Over four decades ago thin-profile and agitated retorting were developed to promote rapid heating to minimize the impact of heat on quality attributes. The retortable pouch has re-emerged as a packaging alternative for both conventional and aseptically processed foods. Aseptic processing and packaging was developed to minimize the heat severity even further by rapid heating and cooling of the food prior to packaging under aseptic conditions to further sustain the nutrient and quality of the food. Quite recently, alternative or novel food processing methods (both thermal and non-thermal) have emerged and are being explored to produce safe and better quality foods. These alternative technologies which include but are not limited to: high-pressure processing, pulsed electric field, pulsed X-ray or ultraviolet light, ohmic heating, radio frequency, microwave, pulsed light, and oscillating magnetic fields could potentially replace conventional thermal processes for some products. The food industry is actively involved in these developments, and poised to adopt new technological alternatives that offer competitive advantages. Each of these alternatives has to be challenged in terms of microbiological capabilities, safety, efficiency and overall quality for acceptance as a main stream technology. This paper focuses on the fundamental principles of thermal processing with emphasis on quality enhancement as it relates to both conventional and alternative technologies that employ heat.

Principles of thermal processing:

Thermal destruction of microorganisms is traditionally established to take place following a first order semi-logarithmic rate.Therefore, theoretically, a sterile product cannot be produced with certainty no matter how long is the process time[1].Targeting a product that is completely void of microorganisms would render the product unwholesome or inferior in quality. Industrially,thermal processes are designed by processing authorities to provide commercially sterile or shelf-stable products. Commercial sterility (as defined by the United States Food and Drug Administration (FDA)) or shelf-stability (U.S. Department of agriculture (USDA)) refers to conditions achieved in a product by the application of heat to render the product free of microorganisms that are capable of reproducing in the food under normal non-refrigerated conditions of storage and distribution. Designing a sound thermal process requires extensive understanding of process methods, the heating behavior of the product and its impact on a target microorganism. Thus, the severity of any thermal process [1] must be known and depend on factors such as:

- The physical characteristics of the food product including thermo-physical properties, shape and size of the container holding the product.
- (The type and thermal resistance of the target microorganisms that are likely to be present in the food.
- The pH, water activity (*a*w) and salt content of the food.Changes in the intrinsic properties of food, mainly

salt, water activity and pH are known to affect the ability of micro organisims to survive thermal processes in addition to their genotype. Due to health-related concerns on the use of salt, there is increased demand to reduce salt levels in foods [2]. The United States Food and Drug Administration (FDA) has classified foods in the federal register (21 CFR Part 114) as follows:

- acid foods
- acidified foods
- lowacid foods.

Acid foods are those that have a natural pH of 4.6 or below. Acidified foods (e.g., beans, cucumbers, cabbage, artichokes, cauliflower, puddings, peppers,tropical fruits and fish) are low acid foods to which acid(s) or acid foods are added with a water activity greater than 0.85 and a finished equilibrium pH of 4.6 or below. Low-acid foods have been defined as foods, other than alcoholic beverages, with a finished equilibrium pH greater than 4.6 and a water activity greater than 0.85. Scientific investigations [3] have revealed that spores of *Clostridium botulinum* will not germinate and grow in food below pH 4.8. To provide sufficient buffer, a pH of 4.6 has generally been accepted as the point below which *C. botulinum* will not grow to produce toxin. Thus, a pH of 4.6 represents a demarcating line between low and high acid foods. During thermal processing of low acid foods (pH≥4.6), attention is given to *C. botulinum*: the highly heat resistant, rod-shaped, sporeformer that thrives comfortably under anaerobic conditions to produce the *botulism* toxin. Commercial sterility is achieved when *C. botulinum* spores are inactivated to satisfy regulatory requirements. However, other heat resistant spores (generally referred to as *thermophiles*) such as *Clostridium thermosaccolyaticum*,*Bacillus stearothermophilus*, and *Bacillus thermoacidurans* have the potential to cause spoilage and economic losses when processed cans are stored under "abuse" storage conditions of temperature. However, *thermophiles* would be of no consequence provided one can guarantee that processed cans would be stored at temperatures below 30 ◦C.

THERMAL PROCESSING OF MILK:

MILK

whole clean lacteal secretion,of the healthy milch animal,,,A perishable product,which require thermal processing for kill of pathogens and inactivate the enzymes which causes negative changes during storage present in it. There are also various types of techniques for preservation of milk,but THERMAL PROCESSING is a most important among them.

The main purpose of heat treatment of milk is to render it sage for human consumption and to enhance its shelf life. Thermal processing is an integral part of all operations/processes of milk and milk products manufacturing units. The common pathogenic organisms likely to occur in milk are killed by relatively mild heat treatment. The most resistant organism is the Bacillus tuberculosis and hence has been made as index organism to achieve complete safety of milk. Any heat

treatment, which may destroy this organism, can be relied upon to destroy all other pathogens in milk. The thermal death of such pathogenic organisms like Tubercle bacilli,Typhus and Coliform bacteria of such pathogenic organisms like Tubercle bacilli, Typhus and Coliform bacteria and Coxiella burnettie (Q fever organism) has made the basis for timetemperature combinations is also a matter of optimization where both microbiological effects and quality aspects must be taken into account. Various categories of heat.

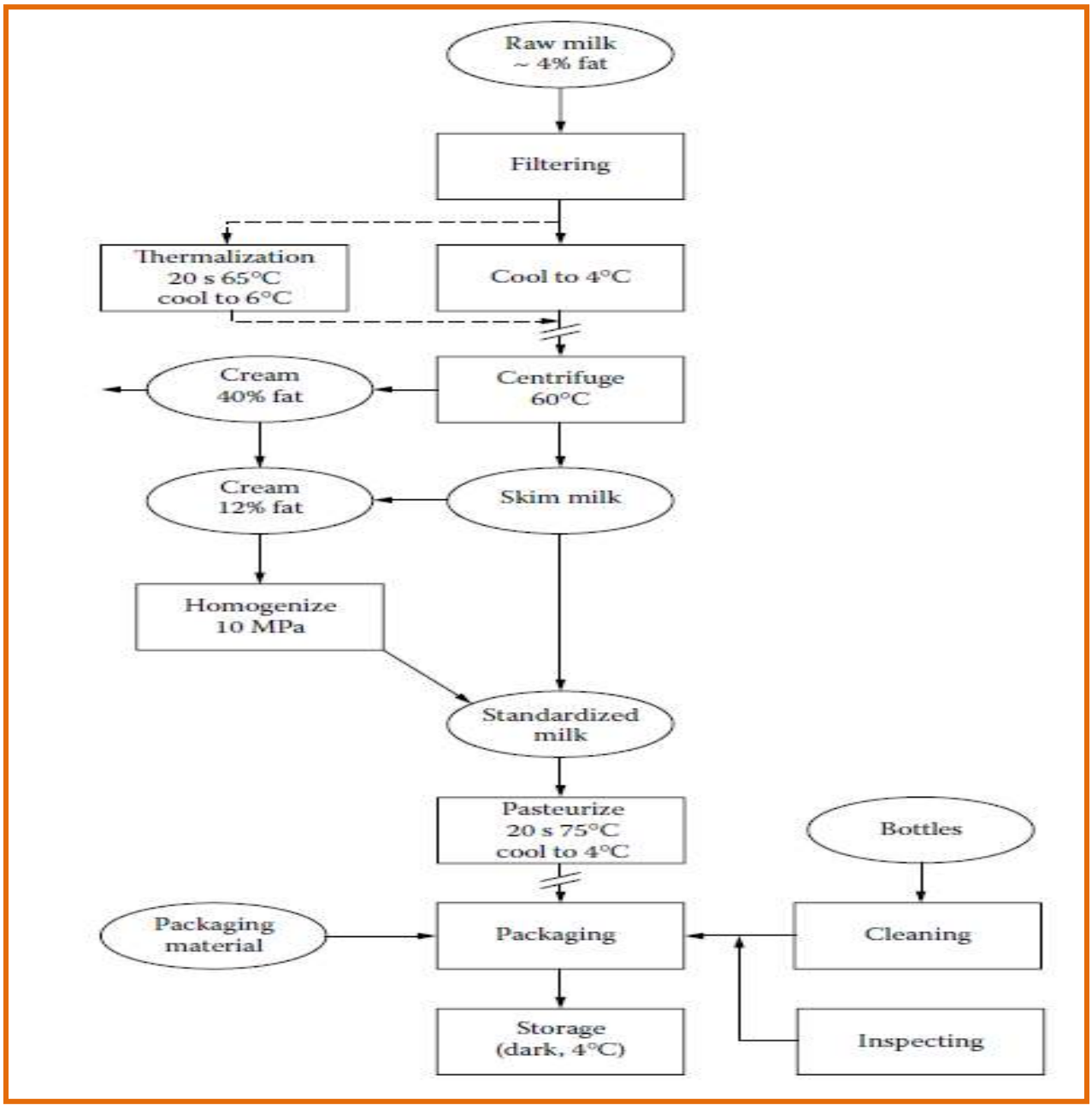
TYPES OF THERMAL PROCESSING OF MILK

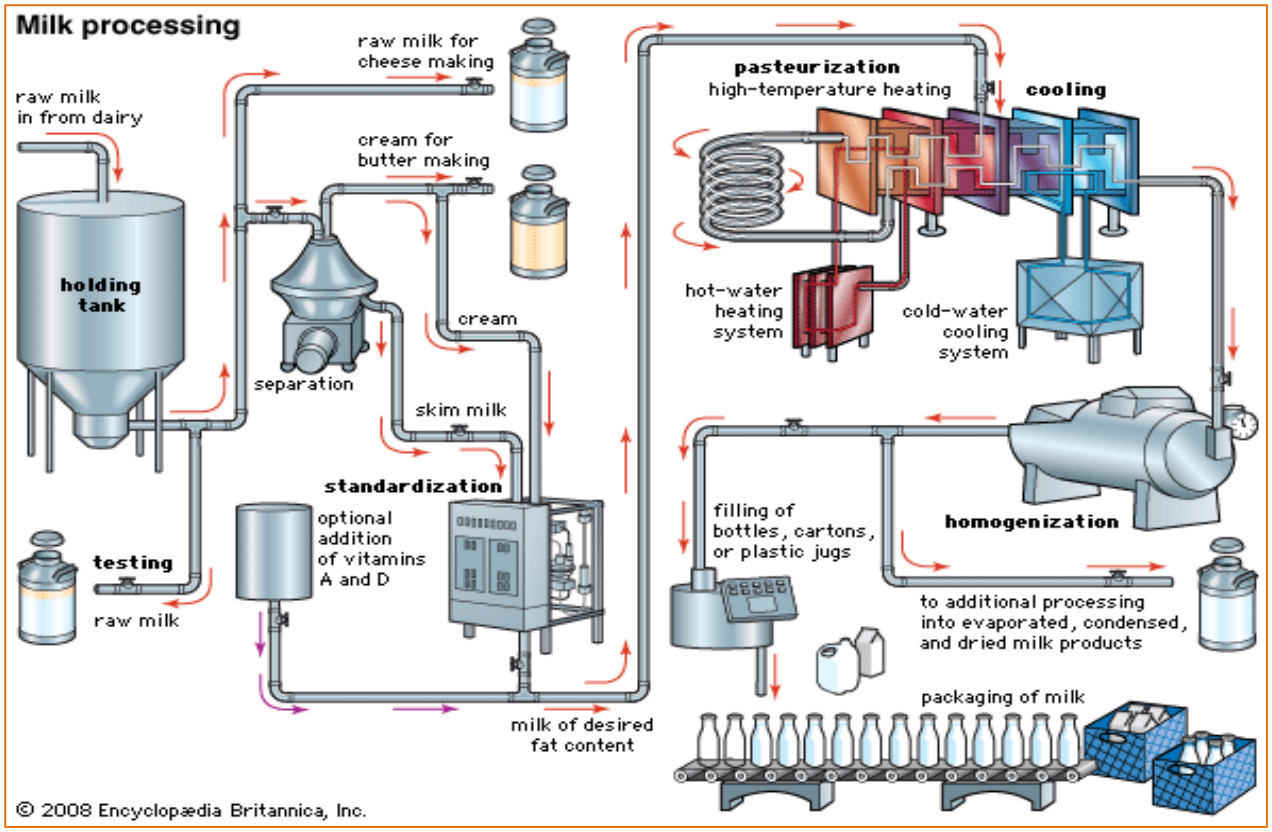
Thermization:
134.6 to 154.4 degrees Fahrenheit (57 to 68 degrees Celsius) for 15 minutes
Batch pasteurization,
low temperature, long time (LTLT): 145.4 degrees Fahrenheit (63 degrees Celsius) for 30 minutes Pasteurization,
High temperature, short time(HTST): 161.6 to 165.2 degrees Fahrenheit (72 to74 degrees Celsius) for 15 to 30 seconds
Ultra hightemperature (UHT) treatment: 275 to 284 degrees Fahrenheit (135 to 140 degrees Celsius) for 3 to 5 seconds Incontainer
Sterilization: 239 to 248 degrees Fahrenheit (115 to 120 degrees Celsius) for 10 to 20 minutes

DIFFERENT CATEGORIES OF HEAT TREATMENT

TREATMENTS	PROCESS	TEMPERATURE(◦C)	TIME(SECONDS)
Pasteurization	LTLT	63	1800
	HTST(milk)	72	15-20
	HTST(cream)	>80	15
Thermization	•	57-68	15
Sterilization	•	115-121	180-780
Ultra-pasteurization	•	115-130	24
UHT	•	135-150	1-6

FLOW DIAGRAM OF THERMAL TREATMENT OF RAW MILK IN A PLANT





THERMISATION:

Mildest of the heating process.134.6 to 154.4 degrees Fahrenheit (57 to 68 degrees Celsius) for 15minutes.Done after platform test ,filtering or clarification of raw milk in a plant. This process consists of heating milk below pasteurization temperature to temporarily inhibit bacterial growth. The process is useful where it is not possible to immediately pasteurization all the milk and some of the milk needs to be stored for hours/days before further processing. The milk is heated to 63-65oC for 15 seconds and rapidly chilled to 4oC or below to prevent aerobic spore forming bacteria from multiplying after thermization. Thermization has favourable effect on spore forming bacteria to revert to vegetative state which are destroyed upon subsequent pasteurization.

PASTEURIZATION:

It is the process of heating every particle of milk or milk products, in properly designed and operated equipment to specified temperature and holding at that temperature for specified period of time followed by immediate cooling and storing at low temperatures.

Term pasteurization coined after the name of LOUIS PASTEUR of France who demonstrated that heating wine at temperature between 50°c -60°c, killed spoilage organisms, helped in preservation.

Pasteurization can be achieved either by holding method (batch process) or continuous process. Under batch process the milk is heated to 63oC for 30 minutes

in a double jacketed vat. Heating and cooling is done by spraying or circulating hot water /steam of chilled
water between the inner and outer jacket of the vessel. The milk is kept gently agitated mechanically to ensure uniform heating/cooling. The process is called **low temperature long time (LTLT) method**. This method is suitable for small quantities ranging from 200-1000 litre requiring low initial cost of equipment.

High temperature short time (HTST) treatment for pasteurization of milk refers to heating every particle of milk in a continuous flow to a minimum of 72oC for at least 15 seconds followed by immediate cooling to 4oC. The entire process is automated and is ideal for large scale handling of 5,000 lph or higher. The complete process of preheating, heating, holding, precooling and chilling is completed in a plate type heat exchanger mounted on a compact frame with inter connected sections to make the process continuous. The heat exchanger plates are so designed as to prevent mixing of thin channels of product and heating/cooling medium by separating the plates with rubber gaskets. The complete equipment consisting of four sections is called pasteurizer. Each section consists of varying numbers of plates depending on equipment capacity. The raw cold milk (4-5oC) from balance tank enters the pre-heating/pre-cooling (regeneration) section, where hot pasteurized milk (72oC) flows counter current to the raw cold milk, within adjacent plates, thereby, transferring heat for pre-heating of raw milk and precooling of pasteurized milk resulting in energy saving. The pre-heated milk then enters the heating section where it is heated to a temperature of 72oC, using hot water or steam, passes to holding section where the temperature of milk is maintained for specified period of time (15 seconds) until it leaves the section. A flow diversion value is placed at the outlet of holding section that senses the temperature and accordingly diverts the milk either forward or returns to balance tank if not properly heated. The pasteurized milk thus passes to regeneration section followed by cooling section where it is chilled using chilled water or glycol solution as a coolant.

OBJECTIVE:

Rendering milk for human consumption by destroy all pathogens.

Improving keeping quality of milk by destroy spoilage organisms at about 85-99%.

PROBLEMS:

- Used to mask low quality milk.
- Diminishes significantly nutritive value of milk.
- Reduce cream line or cream volume.
- Pasteurized milk not clot with rennet.
- Fails to some what destroy bacterial toxins.

IN-BOTTLE PASTEURIZATION:

Bottle filled with raw milk and tightly sealed with special caps ,held at 63-66°c for 30 minute. then bottles pass through water sprays of decreasing temperature which cool both the product and bottle.

ADVANTAGE: Prevent possibility of post-pasteurization contamination.

DISADVANTAGE:

→Slow transfer of heat.

→Every chance of breakage of bottles.
→Over sized bottles used to allow for milk expansion during heating.
→Special types of water-tight caps used.

BATCH/HOLDING PASTEURIZATION(LTLT)

- Low temperature-long time method
- Heated at63°c for 30 minute and promptly cooled to 5°c.

Three types:

Water jacketed vat: Double walled around the sides, bottom in which hot water/steam under partial.

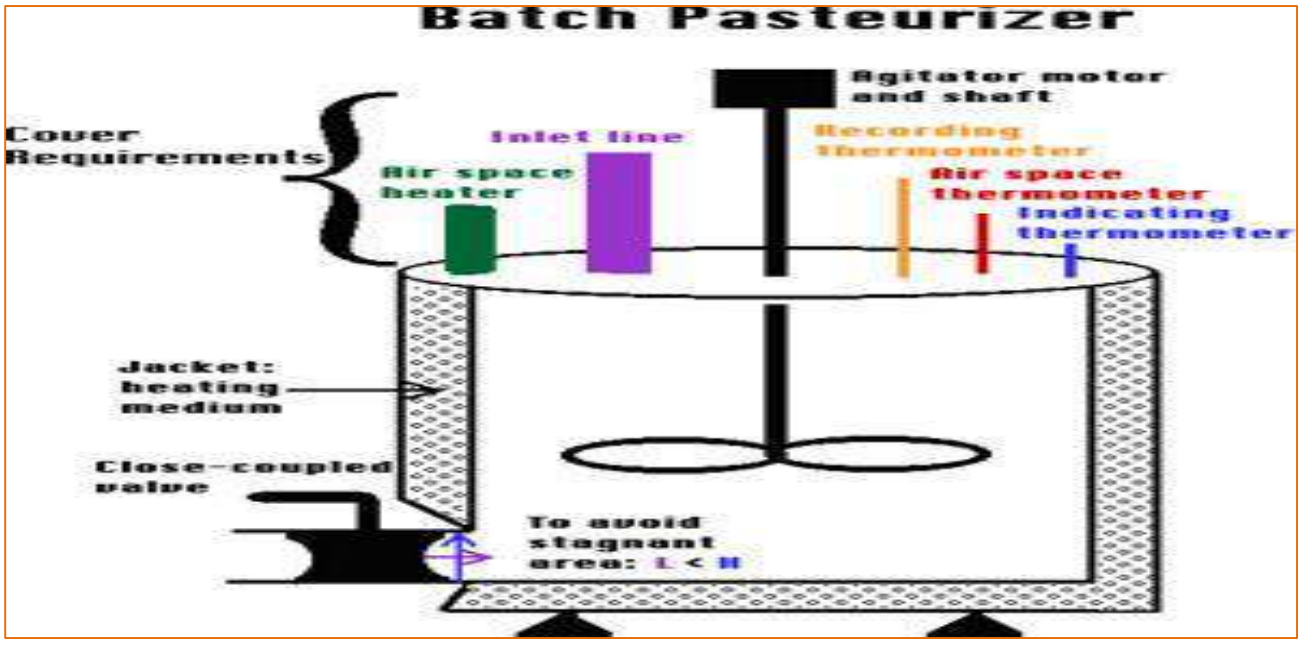
- vacuum circulates for heating, & cold water for cooling.
- Outer wall insulated to reduce heat loss.
- Heat exchange occur through inner lining of wall.
- Agitated by slowly moving propellers. When heating the vat cover is left open for escape off-flavours when holding, cover closed.
- Flexibility in use.

WATER SPRAY TYPE:

→A film of water sprayed from a perforated pipe over surface of tank holding product.

COIL VAT TYPE:

→Heating/cooling medium pumped through a coil placed either in horizontal or vertical position while coil is turned through product.
→Turning coil agitates the product.
→coils difficult to clean.



DISADVANTAGES:

- Not well adopted to handling small quantities of several liquid milk.
- Gaskets require constant attention for possible damage & lack of sanitation.
- Greater accumulation of milk stone in the heating section.(Due to high temperature of heating)
- Need technically qualified staff.
- More destruction of cream line & survival of thermodurics leading to

higher bacterial counts in finished product.

- ❖ Margin of safety in product sanitary control are so narrow that automatic control precision instruments are required its operation.

FACTORS AFFECTING THE EFFECT OF PASTEURIZATION

- ❑ Raw milk quality
- ❑ Processing condition:temperature & time.
- ❑ Post –processing contamination(PPC).
- ❑ Storage-temperature

ELECTRO PASTEURIZATION MILK:

Employs electricity as heating agent,fairly popular in America.

Heated at small specially constructed chamber.It is rectangular,vertical chamber of 2 feet height& 2 inches in cross section with two sides made up of carbon electrode separated by intervening walls of plate glass.Cold milk passes through generative section on which it is preheated about 120°f by outgoing milk ,then pass through the electric heating chamber, here heated at temperature of 161-163°f by resistance offered by milk to passage of a 110-volt altering current. Exposed about 15-20 second.After which it cooled.

Vacuum pasteurization

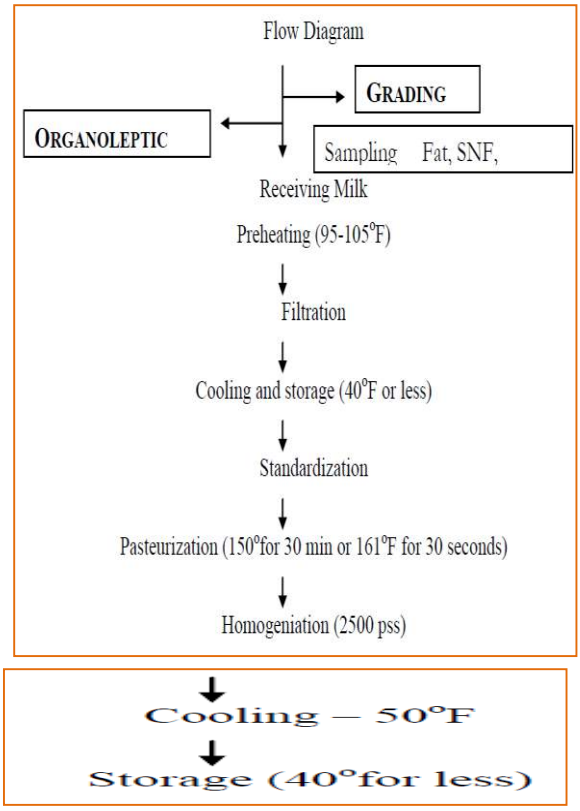
- ➢ By LAMONT MURRAY(1923),Newzealand
- ➢ Cream mixed with steam which both pasteurizes & deodorises.
- ➢ Cream passes through four separate chamber before it dispatched for further processing into butter
- ➢ This pasteurization of milk/cream reduced pressure by direct system.
- ➢ Remove feed &other volatile from cream,and pasteurised for butter making.
- ➢ Vacreator equipment used, so process called as VACREATION.
- ➢ Chamber operated under a vaccum of 5 inches Hg at temperature 90-95°c.
- ➢ Then temperature maintained at71 72°c under vaccum 15 -20 inch Hg.
- ➢ Then maintained at 43°c under vaccum of 26-28 inches of Hg.
- ➢ Time about 10 seconds.

STASSANIZATION:

The milk is heated to about 165oF for 7seconds under slight pressure in a thin layer between two heated surface (in order that all carbonic acid may be returned) the process is carried out in a tubular heat exchanger consisting 3 concentrate tubes by passing milk between two water heated pipes thro narrow space of 0.6 to 0.8 mm. It is claimed that there is practically no milk stone formation, less destruction of vitamins, no evaporation of milk and more economy in steam utilization than in conventional pasteurization. This device was invented on Dr.Henre Stassano. Adv.Easy cleaning.

Ultra –pasteurization.

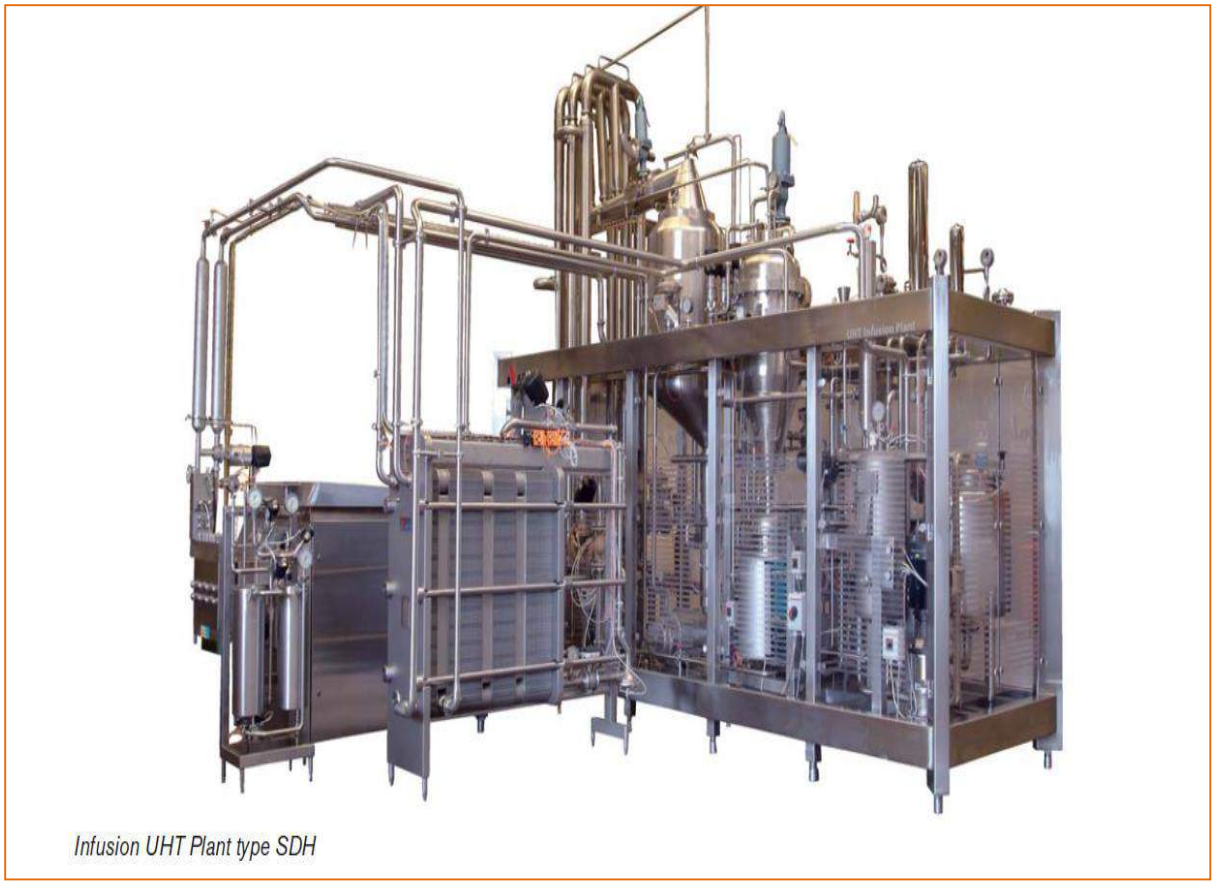
Its objective is to enhance or extend the shelf life of the product (milk) by 15 – 30 days.The fundamental principle is to reduce main causes of reinfection of the product during processing and packaging. This is achieved by heating milk to 115-130oC for 2-4 seconds and cooling it to below 4oC.



This requires extremely high level of hygienic practices to be followed during production and maintenance of temperature lower than 4oC during distribution of such products. Ultra pasteurized products are packed in pre-sterilized containers aseptically and held refrigerated to achieve extended shelf life.

Ultra-high temperature treatment (UHT)

It is a technique for preserving liquid food products by exposing them for brief intenseheating. In short the process is termed as UHT treatment. The heating temperature normally ranges from 135-150oC for 1-6 seconds. The process is continuos which takes place in a closed system that prevent the product from being contaminated by air-borne microorganisms. The product passes through heating and cooling stags in quick succession followed by aseptic filling as an integral part of the process. There exist two methods of UHT treatment indirect heating and cooling in heat exchangers and direct heating by steam injection or infusion of milk with steam and cooling by expansion under vacuum. UHT-treated products are packed asceptically in specially designed multilayer containers, and can be stored at room temperature for extended period of time (2-6 months) without bacterial growth.



Infusion UHT Plant type SDH

UPERIZATION:

- Other wise called "ULTRA PASTEURIZATION"
- Developed in Switzerland.
- Here milk is heated with direct steam up to 150°c for a fraction of a second.
- Continuous process.
- First step in uperization process is for warming of milk at 50°c &intended to remove dissolved oxygen & volatile off-flavour by vaccum treatment.
- In second part milk first preheated about 80-90°c,then heated on uperization chamber with high pressure steam to around150°c for1/2 -3/4th of a second.
- after that move into expansion chamber at room temperature, there by forcing evaporation of moisture.
- then moved to cooler and storage chamber.

ADVANTAGES:

- Look keeping quality.
- Removal of feed and other volatile off-flavours.
- Appreciable homogenization effect.
- Reduction in acidity.
- Efficient destruction of micro organism
- Effect of uperization on nutritive value and flavour are not greater than that of pasteurisation.

BOILING OF MILK:

- Milk temperature at 212.3°F at sea level

- Milk sugar burnt causing a condition called caramelisation(brown in colour}
- Enzymes are destroyed
- Calcium, magnesium, phosphoric salt precipitated
- Thin film formed over the surface by coagulation of small amount of casein, albumin , fat and calcium salt.

Sterilization :

In this process milk or condensed milk packed in clean containers is usually subjected to high temperature (115-120oC) for 20-30 minutes. The containers may be tin cans (200-400 g capacity) for evaporated/sweetened condensed milk or glass bottle for milk. The process of heating and partial cooling is achieved in a rotary autoclave for batch production or hydrostatic tower for continuous production. In container sterilization is the original form of sterilization and is still used.

IRRADIATION:

- Very short exposure of a thin film of milk to the rays of a mercury vapour lamp or other reliable source of ultra violet radiations.
- Improving food value of milk without diminshing nutritive value.
- Develops anti-rachitic properties when exposed to irradiation b y increasing vit-D content
- Reduction Bacterial counts about 90%, frequently up to 98%.

Microwave heating :

- It is a novel method of heating, which greatly reduces the effect of heat penetration lag
- associate with traditional process of convection or conduction. Microwaves form part of the
- electromagnetic spectrum (frequency range 915 and 2450 MHz). The heating effect is achieved
- by transfer of energy to a dipole (in water) within the product. The constant movement of dipole
- due to oscillation of molecules generate heat. The high temperature produced in are of high
- water concentration transfer heat to other areas of food not absorbing microwave energy so well.
- Microwave absorption is inversely proportional to the penetration depth as a function of water
- content, salt content and temperature. During microwave heating temperatures at the surface, are
- often lower due to evaporative cooling than at the centre of the product. Conduction effects are
- only the means of leveling out the temperature imbalance due to microwave heating, Microwave
- absorption characteristics change with change in physical phase of the product. In frozen state
- molecules are less free to move and therefore less able to interact with electrical field. As the
- product melts the areas of water and dissolved salts appear which absorb microwaves rapidly.

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Repeat Breeding: Causes and Diagnosis

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Repeat breeders are cows that are cycling normally, with no clinical abnormalities, which have failed to conceive after at least two successive inseminations Repeat breeding can be a major factor involved in infertility. A "repeat breeder" is generally defined as any cow that has not conceived after three or more services associated with true estrus (heat). A repeat breeder is generally defined as any buffalo that has not conceived after three or more services associated with true estrus. A repeat breeding animal has normal or nearly normal estrus; estrus cycles as well as reproductive tract and though has been bred three or more times by fertile bull semen but had failed to conceive. In herds of normal fertility, where conception rates are commonly at 50-55%, about 9-12% of the cows are expected to be repeat breeders. As the conception rate decreases, the number of cows requiring additional services increases. As a result, repeat breeding rapidly becomes a significant problem. As with other reproductive problems the key to identifying or confirming a repeat breeding problem lies in a good set of records. By keeping and analyzing good estrus and breeding records one can calculate the percent of repeat breeders in a herd. It is important however, when evaluating the significance of repeat breeding in an infertility problem, to keep the definition of a repeat breeder in mind, so only cows requiring more than three services are considered. If natural service is used on the farm, frequent pregnancy exams will be especially helpful in identifying repeat breeders. In general, if more than 15% of the cows require more than three services, repeat breeding should be considered a significant problem warranting further investigation.

***Causes of Repeat Breeding*:**

When repeat breeding is a real problem the first step in correcting it is to diagnose its cause or causes. Unfortunately this can be a difficult task since many factors can, and frequently do, contribute to a failure to conceive or maintain pregnancy. Furthermore, the cause may be a herd problem or a variety of individual cow problems. Herd problems are by far the most common, and those most often causing repeat breeding include:

Inadequate estrous detection, resulting in:

- Improper timing of insemination in relation to the onset of standing estrus
- Cows being inseminated that have not actually been in estrus

***Semen and insemination techniques*:**

- Inadequate semen quality
- Insufficient numbers of sperm
- Improper insemination techniques
- Infertile bull

Cow factors:

- Metritis and/or endometritis (uterine infections)
- Cervicitis and/or vaginitis (cervical/vaginal infections)

Individual cow problems also can cause repeat breeding. Although they are less common and usually not a major factor, they are a part of the problem and cannot be overlooked. Metritis, endometritis, cervicitis and vaginitis can be individual cow problems as well as herd problems.

Other common cow problems include:

- Endocrine (hormonal) disorders
- Cystic ovaries (may also cause irregular or short cycles)
- Delayed ovulation
- Ovulation disorders (these may also be hormonal)
- Obstructed oviducts
- Defective ova
- Anatomical defects of the reproductive tract
- Early embryonic death (may also cause abnormally long cycles)

Diagnosis:

It is necessary to diagnose the aethiology of reproductive failures in cows having an apparently normal clinical history and then, reduce the economical impact. However, although many diagnostic tools are available, it is usually difficult to get a correct diagnostic because sometimes it is unprofitable.A very comprehensive analysis of the entire reproductive program is necessary to effectively diagnose the complete cause of a repeat breeding problem. To accomplish this most successfully all parties involved in the reproductive management program, namely, the producer, inseminator and veterinarian, need to evaluate the problem and review the herd records together First of all, a complete clinical history should be obtained at herd and individual level. Age, parity, milk yield, previous diseases, reproductive indexes, estrous cycles characteristics, insemination schedule, bulls, estrus detection, hormones, food and farm hygiene should be registered. Now, anatomy, morphology and function of cows should be inspected. The reproductive status of animals must be according to their production. Sexual behavior must be evaluated to detect disorders, as muscle or claw lameness. Similarly, it is necessary to examine the behavior of bull and bull-cow interactions when natural breeding is carried out. Vulva, vagina, cervix, uterus, fallopian tubes and ovaries must be evaluated to diagnoses reproductive defects.

When a group of repeat breeders that lack anatomical defects are bred, approximately 25% will become pregnant to a single service. In approximately 15% of the cows, ova are either missing or ruptured. Ova are not fertilized in 25 to 35% of the cows and early embryonic mortality occurs in the other 25 to 35% of the cows. Thus most repeat breeders are not sterile, rather they suffer from lowered fertility.

External inspection and vaginal evaluation

External inspection can identify congenital or acquired anatomical defects as pneumovagina, vulvar defects, tumors or injuries. The anatomy of the area, secretions around vulva or tail, and vulvar and vaginal coloration should be evaluated. Vaginoscopy is helpful to visualize the vaginal cavity and cervix

Rectal palpation in cow

Rectal palpation is a widely used diagnostic method in cattle with high accuracy, easy to be implemented and at low cost in comparison with other sophisticated techniques. The cervix is presented as a solid structure, tubular, fibrous, with 3-4 folds projected inside and localized on pelvis floor in normal non-pregnant cows. Ovaries are located ventrolaterally to the pelvis floor, and sometimes placed under the bone. During anestrus, ovary size ranges from 2 to 3 cm approximately. Follicles (at different stages of growth) and CLs (hemorrhagic, mature or/and albicans) are developed at the ovaries and its size could suggest some diseases.

Ultrasonography :

More recently, ultrasound technology has been used for developing more effective superovulation, embryo collection and recipient´s synchronization. Foetal sex can be determined by ultrasonography (US) from day 50 onward, emphasizing that it can be accurately established around day 60 of pregnancy.Other ultrasound application is the aspiration of oocytes, or "ovum pick-up" (OPU). It is less traumatic and invasive than laparoscopy, does not affect the ovarian activity and can be given many other utilities. Transrectal ultrasound diagnosis has improved our ability to assess the reproductive organs in cattle and to follow the dynamic interactions between ovarian follicular cohorts. Even 2-3 mm follicles can be seen, quantified and sequentially monitored, allowing the development of superovulation regimens, an essential practice for the embryo transfer industry.

Hormonal function tests:

Progesterone can be considered as a sensor of the reproductive capacity, both for its information about the estrous cycle and for its easy determination. Progesterone assay is an objective and accurate test to evaluate the ovarian function and to diagnose certain diseases that otherwise could not be correctly determined, such as delayed ovulation, persistent luteal activity, ovarian cysts or suprabasal progesterone levels. if progesterone is measured around day 19-21 after AI, it could be an effective method to detect females with pregnancy failure. Radioimmunoassay (RIA) and enzyme immunoassay (ELISA) are the usual analytical techniques for determining steroids in biological fluids.

Oviductal patency

The determination of oviductal permeability is interesting, although it is difficult to carry out. An injection of 500 ml of sterile solution containing 30 gr of starch has been described by Kessy & Noakes (1979). It is intraperitoneally injected and starch reaches the oviduct and descends to the cervical mucus if the fallopian tubes are permeable. It takes about 12 hours to arrive at the cervical mucus, where it will remain between 2 and 4 days. A sample of mucus is collected, stained with lugol and observed under the microscope. Other

procedure to study the oviducts individually is infusing phenolsulphonfthalein into the uterine horn. A Foley catheter is inserted through the uterus and the balloon is inflated on the end of the horn before dye is infused. If the oviduct is normal, the dye will cross it, reach the abdominal cavity, and is eliminated by urine. Bladder is catheterized 20 minutes later and www.intechopen.com Clinical Approach to the Repeat Breeder Cow Syndrome 351 urine turns to reddish if oviduct is normal. After 4 hours, the test can be repeated in the other oviduct . Another diagnostic technique to check the oviductal patency is collecting oocytes or embryos, either with or without superovulation treatments. In addition, if they are collected, it is possible to transfer them to cows without reproductive problems, in which case it also becomes a therapeutic tool to overcome this syndrome.

Endometrial cytology and uterine bacterial culture

Infectious diseases could provoke vulvitis, vaginitis, cervicitis or endometritis, and it is interesting to diagnose these disorders. Usually, uterine inflammatory disorders begin with bacterial contamination into the uterine lumen, and continue with adhesion of pathogens to the mucosa, colonization or penetration of the epithelium, and/or release of endotoxins. Uterine inflammation, even in the absence of active bacterial infection, may disrupt embryonic survival and provoke condition . The endometrial bacteriological diagnosis is interesting to detect pathogens implicated in infertility. In cattle, especially due to the cervical anatomy, samples can be taken using a catheter connected to a syringe containing 30-60 ml of sterile saline. It is deposited into the uterus and then it is removed and cultured. Clinical or subclinical endometritis could be diagnosed.

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