

Vets Review



March 2020 / Vol V / Issue I



Progressive Veterinary Doctors' Association



Vets Review

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March 2020 Vol. V Issue I

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Editorial Message

Dear Doctor,

It gives me immense pleasure to wish you Happy New Year 2020 to all our readers/members and veterinary professionals.

The livestock sector in India plays a major contributor to the agricultural economy of our country, not merely in terms of income but also in terms of livelihood and employment. It is usually said that livestock wealth is more equitably distributed than agricultural land. There is an upwards flow of demand for livestock products due to increase in population, growing per capita income and better living standards.

A large number of farmers in India depend on animal husbandry for their livelihood. In addition to supplying milk, meat, eggs, wool and hides animals i.e. cattle, buffalo, sheep, goat, pig etc. are the major source of power for farmers. Thus animal husbandry plays an important role in the rural economy. In this context 'Vets Review' plays as a precursor for the upliftment of animal husbandry sector as well as helps veterinarians to disseminate their scientific knowledge for better management of animal health. We think this is an opportunity for us to renew its usefulness and the value that it will try to provide our readers. We therefore encourage you to write to us with suggestions on how to make it better.

On this hopeful note, we were in our happy journey and let us try to convert it to a National Journal.

Editors

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রাষ্ট্রমন্ত্রী

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ক্ষুদ্র, ছোট ও মাঝারি উদ্যোগ এবং বস্ত্র দপ্তর (বস্ত্র ও তাঁত)
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DATED : 20/02/2020

MESSAGE

I AM HAPPY TO LEARN THAT PROGRESSIVE VETERINARY DOCTORS' ASSOCIATION IS BRINGING OUT THE 5TH ISSUE OF ITS SCIENTIFIC MAGAZINE "VETS REVIEW" ON 01.03.2020 ON THE EVE OF THEIR 6TH ANNUAL GENERAL MEETING AT WEST BENGAL VETERINARY COUNCIL HALL, BELGACHIA, KOLKATA IN A BEFITTING MANNER.

THE MAGAZINE WILL SERVE AS A MIRROR REFLECTING THE VARIOUS SCIENTIFIC ADVANCEMENT, ASPIRATIONS AND NEW INITIATIVES IN THE FIELD OF VETERINARY SCIENCES WHICH I BELIEVE TO BE A VALUABLE INSIGHT FOR ALL VETERINARIANS. I AM SURE THAT THIS EFFORT WILL BE REPEATED OVER THE COMING YEARS.

I EXTEND MY GREETINGS AND BEST WISHES TO THE ENTIRE MAGAZINE TEAM AND WISH THE ENDEAVOURS VERY BEST.

(SWAPAN DEBNATH)

TO
THE GENERAL SECRETARY
PROGRESSIVE VETERINARY DOCTORS' ASSOCIATION



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Vice-Chancellor

No. : VCS/WBUAFS/M-5/52/A
Date : 10.2.2020

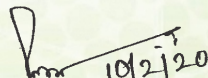
MESSAGE

I am very happy to learn that the 6th Annual General Meeting of the Progressive Veterinary Doctors' Association will be held on 1st March, 2020 at West Bengal Veterinary Council Hall, Belgachia, Kolkata. It is also learnt that a large number of Veterinary Doctors/Scientists and other dignitaries will congregate during this occasion.

I hope that this occasion will throw up opportunities not only for discussing the developments achieved in the field of Veterinary Sciences but also for seeking ways and means to tackle the problems faced by the Veterinary practitioners during their field work.

I am also happy to learn that to commemorate the occasion, the Association is going to bring out the 5th Edition of the Scientific Magazine "Vets Review". I hope that the edition of the Magazine will be informative and helpful for the Veterinary community.

I wish all the best for the success of the 6th Annual General Meeting of the Progressive Veterinary Doctors' Association .


(Purnendu Biswas)

Dr. Subal Chandra Patra
General Secretary
Progressive Veterinary Doctors' Association
37, Belgachia Road, Kolkata- 700037

No. 1/Advisor/2020

Dated: Kolkata, the 25th February, 2020

Message

I am glad to learn that the Progressive Veterinary Doctors' Association has taken the initiative to publish the 5th edition of Technical Bulletin titled "Vets Review" on 01.03.2020 in a befitting manner;

I hope, this bulletin having good quality scientific publications, will be very helpful for all veterinarians in promoting their professional efficiency.

I am also sure that such endeavour will continue over the coming years.

I convey my best wishes for every success of "Vets Review".

(Signature) 25.02.2020
Dr. (Capt.) A.G. Bandyopadhyay

Advisor & Ex officio Principal Director

Directorate of Animal Resources and Animal Health

Government of West Bengal

Dr. Subal Chandra Patra

General Secretary, PVDA

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প্রাণী সম্পদ ও প্রাণী স্বাস্থ্য অধিকার

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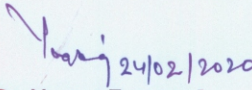


MESSAGE

I am truly delighted to note that the Progressive Veterinary Doctors' Association is going to publish the fifth edition of their Scientific Magazine, VETS REVIEW while observing their sixth Annual General Meeting on 1st March, 2020 at West Bengal Veterinary Council Hall, Belgachia.

Hopefully it will contain good quality scientific publications with field oriented problems and enrich the professional knowledge and efficiency of the veterinarians which would eventually contribute to the development of the existing Animal Resources of our state.

I take this opportunity to extend my hearty wishes for the success of the upcoming edition of the VETS REVIEW along with an effective Annual General Meeting of the Progressive Veterinary Doctors' Association.


[Dr. Yograj Tamang]

Director of Animal Husbandry &
Veterinary Services, West Bengal

To
The General Secretary
Progressive Veterinary Doctors' Association

কার্যালয় : প্রাণী সম্পদ ভবন, তৃতীয় তল, এল.বি.-২ ব্লক, সেক্টর-৩, লবণ হ্রদ, কলকাতা-৭০০ ১০৬

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PREFACE

This is our great pleasure to inform you that scientific wing of Progressive Veterinary Doctors' Association with their utmost venture and enthusiasm could finally publish the 5th edition of Vets Review.

Progressive Veterinary Doctors' Association from the very beginning of its formation, takes pledge to work for the upliftment of Scientific Mindset of our fraternity. We strongly believe that our bulletin is a good platform for exchange of scientific thoughts on animal production and animal health among veterinarians holding different responsible chairs including laboratories, dispensaries, administration, teaching, research and development and extension activities.

Therefore, on the auspicious occasion of 6th Annual General body Meeting of PVDA held on 1st March' 2020 at West Bengal Veterinary Council Hall, Belgachia, Kolkata, we are going to publish the 5th edition of our technical bulletin. The current issue is enriched with reproductive disorders of animals, brucellosis and its vaccination strategy and canine babesiosis at a glance. These reviews have potential to lead for improvements and explorations in the diagnosis and control of animal diseases.

I would like to thank our scientific magazine committee who has taken such good effort to publish this edition in time. We hope that authors, colleagues, and readers could appreciate all the time and effort expended in preparing this issue.

With thanks

Subal Chandra Patra
(Dr. Subal Chandra Patra)

General Secretary

Progressive Veterinary Doctors' Association

REPRODUCTIVE DISORDERS IN BOVINE

Dr Durgadas Mandal and Dr Pradip Sarkar

Assistant Professor

Department of Veterinary Gynaecology & Obstetrics
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Regular breeding depends upon the normal function of the reproductive system. In order to breed regularly, the female has to have functional ovaries, display estrus behavior, mate, conceive, sustain the embryo through gestation, calve, and resume estrus activity and restore uterine function after calving. Each of these functions can be affected by management, disease and genetic makeup of the animal. Impairment in reproductive tract function affects the calf per year programme in bovines.

Fertility: The term fertility as applied to the female denotes the desire and ability to mate, the capacity to conceive and nourish the embryo and finally the power to expel a normal young one and fetal membranes.

Infertility: Sometimes considered as synonymous with sterility or it implies a failure or delay in producing the annual live young one. The term sub fertility is a more appropriate term.

Sterility: Sterility refers to absolute inability to reproduce.

The reproductive diseases or disorder can be broadly classified into three categories

- A. Anatomical or Structural defects
- B. Functional Defect
- C. Infectious Causes

A. ANATOMICAL / STRUCTURAL DEFECTS OF THE REPRODUCTIVE TRACT THAT AFFECT FERTILITY

Anatomical or structural defects are congenital or acquired. Both congenital and acquired

abnormalities of the genital system can influence fertility. Anatomical abnormalities usually affect individual cows or heifers and therefore may not influence the fertility of a herd. In some cows, because of the severity of the abnormalities, sterility is manifested at the time of first service period while in some, where the defect is less severe, it may not be detected until late in life.

Congenital Abnormalities of reproductive tract and ovaries: Aplasia and hypoplasia of the ovaries, segmental aplasia of Mullerian ducts and Imperforate hymen, congenital lack of endometrial gland, double external os of the cervix, Uterus didelphys, abnormal Wolffian Gartner's ducts, Intersexuality or Hermaphrodite and Freemartinism are the example of Congenital abnormalities or defects of reproductive tract.

Acquired abnormalities or defects of reproductive tract: Tumours of the ovary, Ovaritis or infection of the ovary, Para Ovarian cyst, Ovarobursal adhesions, Hydrosalpinx and Pyosalpinx, Adhesion of the Uterus and Postpartum trauma of the Tubular genital tract, Endometritis and Pyometra, Mucometra or Hydrometra, Perimetritis and Parametritis, Abscess of the uterine wall are the example of Acquired abnormalities or defects of reproductive tract.

Congenital Defects:

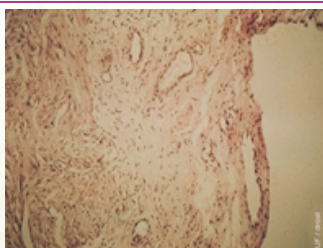
Aplasia of ovary: Means complete lack of development of the ovary. It can be unilateral or bilateral. If bilateral, the animal will be sterile. In case of unilateral aplasia the animal may reproduce but the animal will be infertile and it is not economically viable to maintain such an animals.



Ovarian hypoplasia: Ovarian hypoplasia caused by a single autosomal gene with incomplete penetration is a condition in which one or both ovaries are small, narrow and functionless. The affected ovary may be partially or totally hypoplastic and undergoes incomplete development as a part or whole lacking the normal number of primordial follicles. Depending on the severity of the hypoplasia and whether condition is unilateral or bilateral, infertility or sterility will result.



Segmental aplasia of the Mullerian ducts and Imperforate hymen: Segmental aplasia of the Mullerian ducts and imperforate hymen are developmental defects of the Mullerian ducts that lead to various anomalies of the vagina, cervix and uterus. A single, recessive, sex-limited gene with linkage to the gene for white coat color is considered to be the cause for this condition.



Congenital lack of endometrial glands: This condition has been observed in few heifers where they exhibited failure of estrus and a retained or persistent corpus luteum apparently due to a failure of the endometrium to produce the PGF2 α required for regression of the corpus luteum.



Double external os of the cervix: Double external os of the cervix in cattle is due to failure of the Mullerian ducts to fuse. The lesion often occurs as a band of tissue 0.5 to 2 inches in width and 0.5 to 1 inch in thickness, caudal to the external os of the cervix. On speculum examination it may appear as if there were a double os. In other cases there may be a true double external os of the cervix and the band of tissue separating the 2 openings may extend a short way into the caudal part of the cervical canal. These seldom interfere with conception. Affected cows usually conceive and calve normally. Occasionally a portion of the fetus will pass on either side of the band of tissue and result in dystocia that is easily relieved by incising the band.



Uterus Didelphys: In true uterus didelphys with a double cervix, conception may be delayed when the semen is deposited in the cervix opposite of the ovary from which ovulation has to take place. Since, only one horn takes part in the placentation of the fetus, abortions, premature births, retained placenta, and infertility are more common.

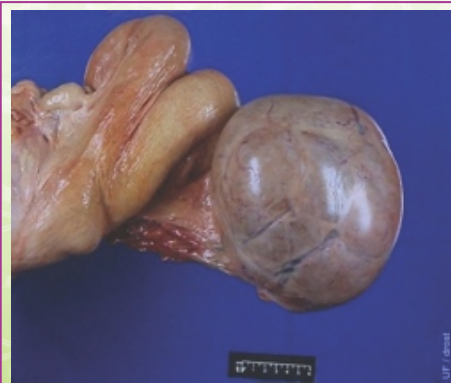


Abnormal Wolffian or Gartner's ducts: Multiple cysts may develop along the course of the ducts or the duct may form a long, sometimes rather coiled, cord 0.5-1.5 cm in diameter distended with fluid.



Intersexuality and Freemartinism: Freemartinism is a distinct form of intersexuality which arises as a result of a vascular anastomosis of the adjacent chorioallantoic sacs of heterozygous fetuses in twin pregnancies. As a result, although the external genitalia of freemartin heifers appear normal the internal genitalia frequently show masculinization.

ACQUIRED DEFECTS OF REPRODUCTIVE TRACT LESIONS OF THE OVARY



Tumours of the Ovary: Granulosa cell tumours are the commonest neoplasm of the bovine ovary but carcinomas, fibromas, thecomas and sarcomas have also been described. Most of the large and cystic neoplasms of the bovine ovary reported in pregnant as well as non-pregnant cattle are granulosa cell tumours. In the early stages of the tumour it presumably secretes estrogen, for the affected animal is often nymphomaniacal. Later, most of the tumour tissue undergoes luteinization and then anestrus usually occurs. Virilism has been reported in long-standing cases.

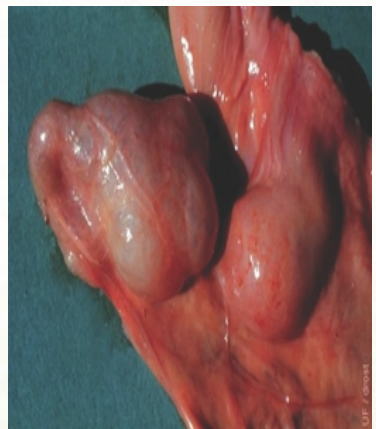


Ovaritis, Inflammation or Infection of the Ovary: Inflammation or infection of the ovary may occur secondary to an ascending infection from the uterus by extension of infection through the uterine walls causing a peritonitis and perimetritis, trauma produced by rough handling or massage of the ovary. Eucleating the corpus luteum or manual rupturing of cysts may lead to formation of star shaped or transverse scars on the ovary.



Para Ovarian Cysts: Paraovarian cysts, vestiges of the Wolffian or Mullerian duct system have been occasionally found in the broad ligament of the cow around the ovary and oviduct but reports are less common in the cow when compared to dog, sheep and horse.

They may be 0.5 to 2 or more inches in diameter and round or oval in shape located most commonly near or in the fimbria of the oviduct.



Ovarobursal Adhesions: Lesions between the ovary and the ovarian bursa are known as ovarobursal adhesions. The extent of the adhesions may vary and may consist of fine web-like strands in the depth of the bursa which does not involve the uterine tube while in others the ovary may be completely enveloped by the bursa. Conception is unlikely to occur due to ovulations from the affected side. Where there are extensive adhesions of the bursa with the ovary, ovulation may not occur and the follicle undergoes luteinization. In some cases ovarian cysts can develop. The condition is rarely seen in heifers but its incidence increases with the age of the cow.



Hydrosalpinx and Pyosalpinx: Hydrosalpinx has been reported secondary to segmental aplasia of the paramesonephric duct or to adhesions of proximal and distal portions of the oviduct. The oviduct gets distended to a diameter of 0.5 to 1 cm or more with clear watery mucus and may appear as elongated, coiled, thin walled and fluctuating on palpation. Pyosalpinx, commonly associated with extensive adhesions of the mesosalpinx and mesovarium may follow severe uterine infection. The condition is less common than hydrosalpinx. In pyosalpinx, extensive perimetritis is not unusual.

LESIONS OF THE UTERUS



Adhesions of the Uterus and Parturient Trauma of the Tubular Genital Tract:

A troublesome sequel to the caesarean operation is adhesion of the uterus to the omentum, rectum, intestines or abdominal wall which is frequently associated with sterility.

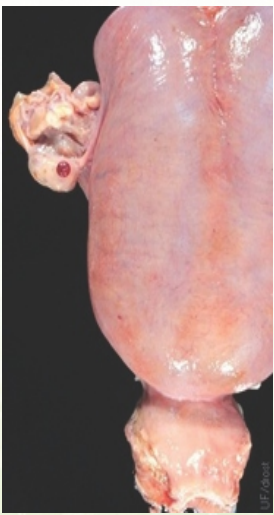
Dystocia due to fetal oversize is common in cattle, particularly in the Friesian breed. Delivery of large calves by heavy traction frequently damages the birth canal to such an extent that the animal is rendered sterile.



Endometritis: Endometritis due to uterine infection and secondary inflammation has been shown to be a cause for infertility in cattle. The condition has been dealt in detail in the module on specific and non specific infections of the reproductive tract.



Pyometra: Pyometra in cattle is characterized by accumulation of pus or mucopurulent material, persistent corpus luteum and failure of estrum. The corpus luteum persists due to failure of PGF2 α secretion as a result of severe endometritis. The condition usually follows an abnormal parturition, uterine infection, and delayed involution of the uterus following abortion, premature birth twin birth, dystocia, retained placenta, septic metritis, or post partum metritis



Mucometra or Hydrometra: Mucometra or hydrometra almost similar in condition are occasionally seen in cattle with the mucin present in the uterus varying from a watery fluid to a semisolid mass. The condition usually follows long standing cases of cystic ovaries, cystic endometrial hyperplasia, arrests in the development of Mullerian duct system or segmental aplasia of the paramesonephric ducts and persistent hymen. The genetic or congenital defects may result in distension of both horns with watery, viscous or even rather solid coagulated masses of mucus and cellular debris that may be confused with pregnancy. In these cows the ovaries and endometrium are normal and estrum therefore occurs normally. Cows with mucometra or hydrometra are usually sterile unless the case is a uterine unicornis where pregnancy can occur in the normal horn, or a simple imperforate hymen that can be opened. In cases that are associated with cystic ovaries treatment should be aimed to resolve the cystic ovarian condition. Cases associated with persistent corpus luteum need to be treated with prostaglandins.

A. FUNCTIONAL/ PHYSIOLOGICAL DISORDERS:

Usually affects individual animals in a herd but, in aggregate it constitutes an important cause of infertility, related to endocrinological disturbances influenced by heredity, diseases, nutrition and stress. Functional form of Infertility includes problems like Anestrus , Sub estrus/ silent heat, Ovulatory defects like Delayed ovulation, Anovulation, Cystic Ovarian degeneration (COD). COD was reported to be most common cause of infertility followed by subestrus, anestrus and anovulation / delayed ovulation.

Anestrus : is a condition when an animal doesn't come to heat and is the most common condition affecting fertility in cattle.

a. True Anestrus: is when ovary is non-functional and will be devoid of any palpable structures. Anestrus is most commonly observed after parturition and post service when conception does not occur.

Causes:

Inadequate or lack of pituitary hormone. Malnutrition: This is the most common cause of anestrus. Inadequate level of carbohydrate, proteins, minerals like P, Cu, Co, Fe etc., and vitamins like vitamin A. Chronic debilitating diseases such as heavy Endoparasitism Seasonal influences. Anestrus are more common during the winters.

Treatment:

1. Improved nutrition

Cereals, concentrates and mineral mixtures
Periodic deworming

2. GnRH - Inj. Buserelin (Receptal) - 5 ml I/m.

3. CIDR 1.9 g of Progesterone for 7-12 days + PGF2a during removal of implant. Oestrus occurs 48-96 hours after its removal and the cows should be inseminated at 48 and 72 hours.

4. Norgestomet s/c implant +5mg E2 for 9-10 days. Estrus@2-3 days after removal-AI (48-72hrs later)

b. Anoestrus due to Persistent Corpus Luteum / Pathological Anestrus

Corpus Luteum secretes progesterone, responsible for maintenance of pregnancy. It is functional and persists on the ovary only during pregnancy if not it regresses. If it persists in non pregnant animals it is termed as PCL or Retained CL. Retained CL is often associated with other pathological conditions of the uterus, which causes the uterus to react as gravid uterus.

i) Mummification ii) Maceration iii) Pyometra
iv) Early embryonic death v) Luteal cyst

i)Mummification : It is a condition wherein the fetal fluid and soft tissue is reabsorbed leaving just a mass of bone and skin tightly enclosed by the contracted uterine walls.

Causes:

No definitively known cause. Various theories are: 1. Genetic factor, 2. Infection and 3. Torsion of umbilical cord

More commonly found from 3rd month of gestation. The condition is not diagnosed until the end of gestation period because the animal is in anoestrus due to PCL. Diagnosed by per rectal examination. A tightly enclosed mass of the conceptus can be felt

Characteristic Features In Mummification:

Failure of udder development, Failure of abdominal enlargement , Failure of parturition , Absence of placentomes and fetal fluids , Hard and firm fetus , Absence of fremitus, Presence of corpus Luteum

Treatment:

- Expulsion using traction if cervix is dilated.
- Estrogen inj. daily for 4-7 days until the cervix dilates, if cervix is closed or inadequately dilated.
- Estrogen injection- Stilbestrol 40-80 mg I/m, Estradiol - 5 -8 mg.
- Estrogen brings about following changes, which lead to expulsion of conceptus, uterine contraction, relaxation of cervix and knocks off CL.
- The conceptus and uterus are sterile in mummification.
- Supportive treatments with antibiotics.

ii) Fetal Maceration: Fetus succumbs to bacterial or viral infection resulting in death, emphysema and maceration characterized by abortion or dystocia. Usually results dystocia due to insufficient dilatation of cervix.

Symptoms:

- ❖ Foul, fetid reddish grey Vulval discharge. On rectal examination an emphysematous fetus or macerated bones could be found.

- ❖ On rectal examination in the cow, fetal bones may be palpated in the uterus either floating in pus or crepitating with little pus around them. The uterine wall is thick and heavy and the cervix usually large and hard.
- ❖ History of chronic, fetid, mucopurulent discharge from the vulva over a period of several weeks or months.
- ❖ Generalized symptoms of elevated temperature and pulse and anorexia are usually not present.

Treatment

- The prognosis is poor.
- Treatment in the cow is difficult.
- Bones are attached with endometrium and endometrium is severely damaged.
- Slaughtering the animal is an option.

Retention of Fetal Membranes/Retention of Placenta:

- Condition where all or part of the placenta or membranes are left behind in the uterus during the third stage of labor.
- In a normal condition, a cow's placenta is expelled within a 3 -8 hour period after calving.
- If placenta is not expelled for 12 hours or more after parturition considered ROP.

Causes of ROP

1. Immature Placentomes: Premature delivery or abortion leads to retention because of immature placentomes.
2. Delayed Gestation
3. Lack of Uterine Contractions
4. Placentitis or Cotyledinitis: Brucella abortus, tuberculosis, Vibrio fetus, IBR, Staphylococcus aureus, E. coli.
5. Uterine Atony: Associated with dystocia, hypocalcaemia, hydropic and other pathological conditions.
6. Severe Deficiency of Selenium, Vitamin A, D, E

Treatment :

- Oxytocin 50-100 IU in cattle/buffalo may be injected.
- Oxytocin is not drug of choice if fetal membranes are retained inside.
- If case is reported late after 24 hours then prime the uterus with estrogen by giving 1-2 ml estrogen and then after 30-60 min give oxytocin.
- Estrogen pulls the fetus and activates the receptors of oxytocin, relaxes cervix.
- PGF₂α is drug of choice in habitual cases but should be given within one hour after parturition.

Manual Removal: Not a preferred method. If placenta is hanging.

Epidural

Separation of Placentome with fingers carefully.

Anoestrus Due to Undetected Heat/ silent heat:

- Inability or ignorance of the owner to detect heat
- The weak heat or silent heat may be attributed to inadequate estrogen level.
- Ascertained only by rectal palpation and detecting CL, ovulation fossa on the ovaries and changes in the reproductive tract.
- Heat stress, nutritional deficiencies.

Treatment:

- Estrogen Inj. at 17th days of the oestrus cycle and repeated on the 20th day.
- Improved nutrition.

Repeat Breeding Syndrome: Cows or heifers, having regular estrous cycles and appears normal on superficial clinical examination, fails to become pregnant following three or more consecutive breeding resulting into long service period and inter-calving interval.

Causes:

1. Anatomical abnormalities

- kinked cervix
- Ovaro-bursal adhesions
- Tumors of bovine reproductive organs

- Persistent hymen
- Persistent Hymen (Rupture during AI)

2. Infectious causes:

- Turbid cervico vaginal mucus indicates uterine infections.
- Unsterilized instruments for insemination and injudicious use of intrauterine medications, lead to endometritis (intrauterine infusion of: antibiotics or Lugol's iodine (0.25%, 20-30 ml, Intrauterine, alternate day two infusions).

3. Nutritional Reason:

- Conception of the animal is related to the body weight(225-250 Kg jersey (250-275kg HF heifer
- Underweight animals always have less chances of conception.
- Strictly follow the deworming calendar/ schedule.

4. Managemental Causes:

- Not inseminated at right time (A.M-P.M rule)
- Double insemination is recommended for crossbreds.

5. Hormonal aberrations:

- Cystic ovarian degeneration (COD) is one of the major causes of RB.
- Delayed ovulation is also associated with this malady (LH).
- Delayed function of CL either alone or in combination with lowered secretion of P4 during luteal phase

6. Poor Semen Quality or Faulty AI Techniques:

- Involves all steps from semen collection to AI
- Correctness and precision of these steps are mandatory in achieving high success rate with AI.

7. Immunological reason:

- Sperm antigens are recognized as foreign material by female's immune system and antibodies develop against sperm antigen
- **Treatment** is just change the bull next time.

Functional Form of Infertility: (Ovulatory defect)

The defects associated with ovulation are:

- Delayed ovulation (Aging of sperm or death of ovum or spermatozoa)
- Anovulation.
- Cystic ovarian disease (COD)/Cystic Ovarian degeneration

Delayed ovulation : Ovulation takes place 48-72 hours after the onset of oestrus but the spermatozoa would be dead by then.

Reasons:

Endocrine reasons: (quantity of LH released is insufficient or its timing is incorrect.

Mechanical reasons: Adhesion of the ovarian bursa to the surface of the ovary. There is no treatment if there are ovario-bursal adhesions. But LH or hCG (Chorulon) @ 3000 IU in adult cattle may be tried.

Anovulation

- As the name indicates the mature Graafian follicle fails to ovulate.
- The animal has normal cycle, normal reproductive tract but fails to conceive. This is due to
 - ✓ Ovaro-bursal adhesion.
 - ✓ Inadequate level or absence of L.H.

Treatment:

- L .H. preparations (HCG- human chorionic Ganadotrphin)- 3000 IU. I/V. when the animal is in heat.
- Inj. Receptal - 5 ml I/m.
- Improve feeding.

Cystic Ovarian Degeneration (C.O.D)/Cystic Ovaries:

Contain one or more persistent fluid filled structures larger than a mature follicle i.e. >25 mm in diameter in one or both ovaries. More

commonly seen in the high producers at around 15-45 days postpartum. Arise as a result of anovulation of a Graafian follicle. Instead of regression and atresia, or luteinization followed by regression, the follicle increases in size, there is degeneration of granulosa cell layer and cyst persists for at least 10 days.

Cyst may be Follicular Cyst or Luteal Cyst

Follicular cyst:

- It is a thin walled fluctuating structure. There may be multifollicular cyst (these cyst may have demarcation between them). Bigger the cyst - more estrogen production.
- structure on the ovary that is at least 25 mm in size in the absence of any corpus Luteum
- Diagnosis is through USG (Very thin outer wall with black fluid extending to its outer edges).

Clinical signs

1. Nymphomania 2. Edematous swelling of vulva, frequent and copious discharge of clear mucus 3. Shortened interval between successive heats. 4. Sterility hump.

Treatment of follicular cysts:

Various approaches to the treatment of ovarian cysts have been used since the disease was first described in 1831.

- Manual rupture: Manual rupture of cystic structures by palpation per rectum. Ovarian haemorrhages and adhesions may follow manual rupture, which could further cause infertility. Therefore, manual rupture should be discouraged.
- Gonadotrophin-releasing hormone (GnRH): After treatment with GnRH analogue (Buserelin), most of the cows that respond come in oestrus 18 to 23 days after treatment. Receptal (Buserelin) - 5 ml I/M.
- Administration of hCG : Chorulon (hCG) - 3000-5000 IU- I/V

- Sequential GnRH and PGF2u treatment: Ovarian cysts that luteinize in response to GnRH administration undergo regression similar to that of normal corpora lutea. The luteolytic activity of PGF2 α reduces interval from the treatment with GnRH to the first oestrus (18 to 23 days). PGF2 α should be given on 9th days, after GnRH treatment.

0 day ----- Receptal----- 5 ml I/M

9 th day ----- Lutalyse-----5 ml I/M

- Administration of progesterone: 50 to 100 mg progesterone (Duraprogen) I/M for 14 days or a single dose of 750 to 1000 mg repository progesterone is used for the treatment of follicular cyst.

Luteal Cyst :

When the wall of follicular cysts gets luteinized, it results in formation of luteal cyst. Progress into luteal cysts by forming a thicker wall of luteal tissue around their outer edges. Structure functions as a persistent corpus Luteum and produces Progesterone. Luteal cysts are never multi follicular i.e. luteal cysts are single on one ovary.

Clinical Signs: If animal remains anestrus for long time, then it shows masculinisation. Although these animals will attempt to mount other cows, but unlike the nymphomaniacal cows they will not stand to be mounted by other cows.

Treatment of luteal cyst:

- The treatment of choice is luteolytic doses of PGF 2 α if a correct diagnosis can be ascertained.

A normal estrus is expected in 3-5 days. The major limitation of this treatment is the difficulty in accurately estimating the amount of luteal tissue present. If the structure being diagnosed as a luteal cyst is really a developing CL, it may not respond because dairy cows do not become highly responsive to the luteolytic action of PGF 2 α until day 6

after estrus. PGF2 α analogue - Lutalyse 5 ml – I/M

- Luteal cysts also respond to human chorionic gonadotropin and GnRH therapy that is effective in the treatment of follicular cysts, but the next estrus could occur 5–21 days after treatment.
- Because of poor estrus detection practices on many dairy farms, the treatment of choice for both follicular and luteal cysts is intravaginal progesterone/prostaglandin.
- Manual rupture of luteal cysts is not recommended because of the risk of trauma and hemorrhage.

Prevention:

- ❖ Selective breeding: The use of only such bulls whose daughters have shown low incidence of ovarian cyst.
- ❖ GnRH treatment on days 12 to 14 post-partum reduces the incidence of ovarian cysts.

C. INFECTIOUS CAUSES OF INFERTILITY:

Bacterial: Bacterial abortions result from brucellosis, leptospirosis, campylobacteriosis (vibriosis), listeriosis, Haemophilus somnus complex, and ureaplasmosis. Bacteria like Salmonella, Actinomyces, Escherichia coli, Streptococcus, Staphylococcus, Bacillus, Pseudomonas, Proteus, Pasteurella, Nocardia, and chlamydia species, as determined by the microbiological findings, can cause abortion. All these organisms and few others that are not listed have been isolated from sporadic cases of abortion. These are secondary to either a septicemia in the dam or ascending infection through the vagina and cervix or due to persistent endometritis.

Brucellosis: Bovine brucellosis is the well known and most controversial infection of the bovine reproductive system. Brucellosis is caused by the bacterium Brucella abortus. The organism has an affinity for certain body

tissues such as the udder, uterus, lymph nodes, testicles, and accessory sex glands. Because of its affinity for the uterus, abortion is the usual sign of the disease. Brucellosis is a particularly difficult disease as there is no sure way to identify infected cattle by their appearance; all infected cattle do not abort. In addition, the incubation period for brucellosis is variable.

Brucellosis is primarily transmitted to susceptible animals by direct contact with infected animals. Aborted fetuses, placental membranes, placental fluids, and the vaginal discharges that persist for several days after an infected cow has calved or aborted contaminate surroundings all around with virulent Brucella organisms. The organism may be transmitted to other animals that contact the environment that has been contaminated with discharges from infected animals. Milk and colostrum from infected cows are the readily available source of infection for calves and the human population. Because brucellosis can easily be transmitted to people, aseptic procedures such as using disposable gloves when examining or assisting cows at calving are highly recommended. Cattle can generally be moved interstate from brucellosis free states without testing. However, cattle moved from non-free states must be tested before moving across state lines. Just because an animal or group of animals has been tested and declared free of infection does not ensure that some are not in the incubation stage of the disease. Therefore, any newly purchased cattle should be quarantined and retested in 45-120 days. This may be the most important part of a preventive program, and one over which the buyer has complete control.

A relatively new RB51 vaccine has replaced the old Strain 19, the only vaccine available for many years. The biggest advantage of the RB51 vaccine is almost total elimination of

false positives observed with use of Strain 19. Use of Strain 19 often resulted in antibody titers that were difficult to differentiate from actual infection titers. Heifer calves can be vaccinated between the ages of 4 and 12 months; about 6 months of age is best. Brucellosis vaccine can only be administered by licensed veterinarians.

Leptospirosis:

Leptospirosis is a contagious, bacterial disease of animals and humans. In cattle, horses, pigs, sheep, goats, and dogs, it has been characterized by a wide variety of conditions including fever, icterus (jaundice), hemoglobinuria (bloody urine), abortion, and death. However, the concept of this disease has recently changed. It is used to be considered a highly fatal disease, but is now thought to be a widespread, mostly subclinical infection of many species of wild and domestic animals range from mild, unapparent infections to acute infections that end in death. Clinical signs that precede abortions may suggest leptospirosis, highest abortion rate occurs in the last 3 months of gestation. Antibodies first appear in the serum of infected animals by the sixth or seventh day, and titers rise rapidly to a high level. Titers then decline to a more or less constant level and may persist for months. Samples sent to the laboratory for culture must be collected and shipped as rapidly as possible because leptospiras do not survive long in dead tissue. Vaccination with killed bacteria protects against clinical leptospirosis for up to a year, but the bacterin must contain the antigens of the strain to which the cattle are exposed.

Campylobacteriosis (Vibriosis):

Campylobacteriosis (vibriosis) is a venereal disease of cattle caused by the organism *Campylobacter fetus* subspecies fetus. Before 1973, this organism was known as *Vibrio fetus* subspecies venerealis, Campylobacteriosis is

characterized by infertility with an increased number of services necessary for conception. Early embryonic deaths are common. In this case, infertility caused by endometritis results in early embryonic death and a prolonged period (up to 120+ days) passes before successful conception occurs. Spread of the organism to the male is primarily by way of copulation with an infected female. A definite diagnosis of genital campylobacteriosis can be difficult and laboratory test results are often disappointing. Although blood tests are available, they are not reliable because it is not a systemic disease and antibodies are rarely found in the blood stream. Most infected heifers rid themselves of the organism within 6 months of sexual rest, thus a reduction of demonstrable antibodies occurs. Bacteriological examination of aborted fetuses appears to be the only practical method of confirming the diagnosis later in gestation. Without vaccination, control and prevention of this disease can be difficult. Both killed and modified-live vaccines are available. Vaccination of bulls has been reported to be effective for both prevention and also as a cure for *Campylobacter fetus* infection. Initial vaccination should consist of two injections before breeding time.

Listeriosis: *Listeria monocytogenes* is a well-recognized cause of abortion, encephalitis and septicaemia in cattle. *L. ivanovii* has also been implicated as a cause of abortion in cattle but occurs less frequently than *L. monocytogenes*. Listeric infections and abortions usually develop in the late winter or early spring. Abortions are most commonly recognized in the last trimester of pregnancy, frequently in the absence of other clinical signs. As well as being recovered from the environment, Listeriae have also been frequently isolated from animal faeces, and various animal sources including meat and

milk from cattle and buffaloes. The faeces of healthy animals often contain *L. monocytogenes* so cross contamination with pathogenic species is likely to occur.

L. monocytogenes and *L. Ivanovii* *L. monocytogenes* and *L. Ivanovii* were isolated from buffaloes, cows, does and ewes with reproductive disorders (endometritis, repeat breeding). The organism is, so far, usually sensitive to a wide range of antibiotics. Ampicillin, amoxicillin, tetracyclines, chloramphenicol, α -aminoglycoside, trimethoprim and sulphamethoxazole are recommended.

Culling of infected animals should be advocated as they secrete the organisms in secretions and excretions, especially in the cases of mastitis. Care in the use and preparation of silage is important as the pathogen grows luxuriantly at a pH lactum antibiotics, together with an greater than 5, particularly when fermentation is ineffective and moulds grow. The silage, which lies within a few inches from the front top and sides of a clamp, should not be fed to animals, especially sheep. So far no vaccine is available against listeriosis.

Mycotic: Fungal or mycotic infection of the placenta is one of the most common causes of sporadic bovine abortion. Providing good health (via good management and nutrition) and avoiding moldy feeds can reduce the incidence. When possible, depending on the availability and demand decrease the period of confinement, decrease cow density, and improve vaccination.

Viral abortion:

IBR (Infectious Bovine Rhinotracheitis or "Red Nose"):

Infectious Bovine Rhinotracheitis virus is the cause of respiratory disease of cattle. However, in cows and heifers, this virus can also cause vulvovaginitis (inflammation of the vulva and

vagina) and abortion. Abortion typically occurs about 20 to 45 days after infection. A diagnosis of IBR-induced abortion is made by laboratory examination and testing of fetal tissues. A blood test may aid in the identification of infected cattle. The control of IBR infections can be accomplished by the use of vaccines.

BVD (Bovine Virus Diarrhea)

Bovine Virus Diarrhoea virus infection can cause abortion, weak calves at birth, calves with brain damage (cerebellar hypoplasia) or other abnormalities of fetal development. Clinical signs in newborn calves infected with BVD can include fever, nasal discharge, diarrhoea and inability to move about normally (ataxia). A diagnosis of BVD virus infection requires laboratory examination of the fetus or calf. A blood test may aid in the identification of infected cattle.

Protozoal

Protozoal diseases causing abortion are Trichomoniasis, Sarcocystosis ("Sarcosporidiosis") and Neosporosis.

Trichomoniasis

Trichomoniasis, is a venereal disease of cattle. It is caused by the protozoan, *Trichomonas fetus*. These organisms are harboured in the reproductive systems of infected animals, and are transmitted from one cow to other cow by infected bulls. Cows will generally get rid themselves of the disease after 60 to 90 days of sexual rest, but infected bulls appear to be unable to develop immunity. Infertility is the most common clinical sign of a trichomoniasis infection. Abortion generally occurs early in gestation (first 3 months). Because little tissue is shed during these early abortions, they often go undetected. Commercial vaccines are now available. Proper immunization requires two injections, usually administered two to four weeks interval. Annual revaccination may be recommended

Conclusion

By only thorough investigations of management and health examination it is possible to detect infertile, sub fertile and sterile animals. There are different aspects such as nutrition, management and infectious agents that affect the infertility. Nutritional aspects includes, green fodder, balanced feeding, mineral

supplementation. Managerial aspects includes breeding policy, timely detection of estrus, care of pregnant animals, care of post parturient dams, vaccination schedule for important diseases. Infections adverse influence which might include bacterial, viral, protozoal and fungal infections affecting genital tracts.

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REPRODUCTIVE DISORDERS ENCOUNTERED IN PRACTICE IN DOGS AND BITCHES

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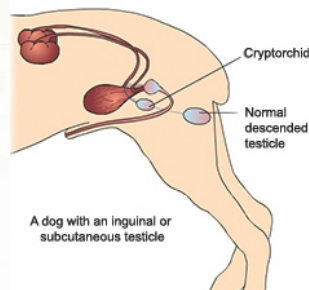
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In practice, reproductive disorders in dogs and bitches hold a major portion of the total number of cases represented. For ease of understanding and for comprehensive idea, the discussion will be divided into male and female disorders separately.

Major Male reproductive disorder:

A. Cryptorchidism

1. Cryptorchidism is a failure of one or both testicles to descend into the scrotum.
2. The condition has a genetic basis and can be inherited from either parent.
3. Cryptorchid dogs should not be used for breeding.
4. Affected animals should be neutered due to an increased risk of developing testicular cancer.



Treatment:

10 mcg of Buserelin acetate, a GnRH analogue, 3 times at weekly interval may be tried for descending the testicles. If not descended, best remedy is removal of the retained testicle.

B. Paraphimosis

1. Paraphimosis, or the inability to completely retract the penis into the preputial cavity
2. Usually occurs after erection.
3. Mostly occur after semen collection or breeding.



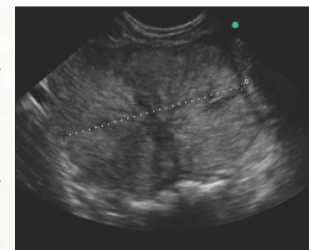
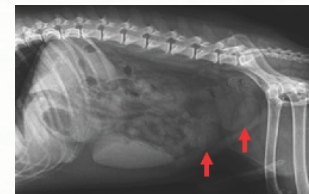
4. Other causes of paraphimosis include trauma to the penis.

5. Paraphimosis is a medical emergency because the exposed penis quickly becomes swollen (due to accumulation of fluid), dry, and painful.

Treatment: The treatment consists of gentle cleansing and lubrication of the exposed penis. The penis is replaced inside the prepuce and the swelling resolves once circulation is restored.

C. Disorders of the Prostate

1. The prostate gland is located within the pelvis behind the bladder.
2. The prostate gland is not required for sperm production, but it is important for successful breeding.
3. The prostate gland provides the major part of the fluid in the ejaculate and is important in nourishing the sperm cells and increasing their movement.
4. Diseases of the prostate gland are common in dogs that have not been neutered, especially enlargement of the prostate (benign prostatic hyperplasia). Other prostate diseases, including bacterial infection, abscesses, cysts, and tumors, are less common and can be seen in neutered males.



Benign Prostatic Hyperplasia

Enlargement of the prostate is the most common prostatic disorder. It is caused by

male hormones. It is found in almost all unneutered dogs over the age of 6 years. There may be no signs, or straining to



defecate, blood in the urine, or preputial discharge may occur. Neutering is the preferred treatment. Reduction in the size of the prostate usually follows within a few weeks of the surgery. In dogs used for breeding, medication to decrease the size of the prostate may be helpful.

Reproductive Disorders of Female Dogs

There are many reproductive diseases that are encountered in clinical practice.

A. False Pregnancy (Pseudopregnancy)

1. False pregnancy (pseudopregnancy) is common in female dogs.
2. It occurs at the end of the heat cycle and is characterized by
 - ✓ weight gain,
 - ✓ enlarged abdomen,
 - ✓ swelling of the mammary glands,
 - ✓ milk production,
 - ✓ behavioral changes.
3. Treatment is often not recommended because the condition usually ends on its own in 1 to 3 weeks.
4. One should not milk out the mammary glands, because this will only stimulate production of more milk.

B. Ovarian Remnant Syndrome

Ovarian remnant syndrome is caused by ovarian tissue that was left behind when a bitch was spayed. This is a complication of the surgery. The most common signs are those of heat (swelling of the vulva, flagging, and standing to be mounted).

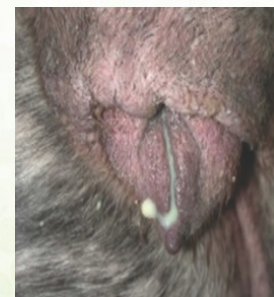
C. Pyometra

1. Pyometra is a bacterial infection of the uterus due to hormonal changes in unspayed bitches.
2. It is reported primarily in dogs more than 5 years old, and tends to occur 4 to 6 weeks after estrus. After estrus, the level of progesterone stays high to prepare the uterus for pregnancy by thickening its surface. If pregnancy does not occur for several cycles, the lining inside the uterus continues to thicken and cysts can form within the uterus.
3. These cysts and accumulated uterine fluids provide an ideal environment for bacterial infection.
4. Pyometra can occur due to administration of estrogen- or progesterone-based medications.
5. The signs are variable and include
 - ✓ lethargy,
 - ✓ poor appetite,
 - ✓ increased thirst and urination,
 - ✓ vomiting.



6. When the cervix is open,

➤ Discharge of pus, often containing blood, is present. When the cervix is closed there is no discharge and the large uterus may cause abdominal enlargement. Signs can progress rapidly to shock and death. The infection is diagnosed by physical examination, determination of the nature of the discharge, ultrasonography, x-rays, and laboratory and blood tests.



➤ Removal of the ovaries and uterus ("spaying") is the recommended treatment in most cases. For younger animals that are not seriously ill and that will be bred in the future, antibiotics, intravenous fluids, and prostaglandin can be administered.

BRUCELLOSIS VACCINOLOGY IN LIVESTOCK

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Introduction :

Brucellosis is a zoonotic disease of domestic and wild terrestrial and marine mammals caused by the bacteria of the genus *Brucella*. The genus *Brucella* belongs to the α -Proteobacteria order and consists of mostly intra-cellular bacteria which are Gram-negative, non-spore-forming non-encapsulated coccobacilli or short rods with rounded ends. Among domestic animals, brucellosis is prevalent in most countries of the world, and primarily affects cattle, buffalo, pigs, sheep, goats and dogs.

As a zoonotic disease, brucellosis is of serious public health significance. The most virulent characteristics of all the brucellae are observed in *Brucella melitensis* infection. In cattle, brucellosis is primarily caused by *B. abortus*.

Species	Natural Host	Zoonotic potential
B. melitensis	goats and sheep	Yes-High
B. abortus	cattle and buffalo	Yes-High
B. suis	pigs	Yes-High
B. canis	dogs	Yes-Moderate
B. ovis	sheep	No reported infections
B. neotomae	desert woodrat	No reported infections

Brucellosis can pose a significant economic loss to owners of domesticated animals due to loss of progeny, reduced milk yield, and infertility. In animals, brucellosis causes epididymitis in males and abortion, placentitis, and infertility in pregnant livestock. In humans, brucellosis causes acute inflammation and manifests many symptoms of a flu-like infection, including undulating fever, sweats, headaches, back pains, and physical weakness. Symptoms of chronic brucellosis include

recurrent fevers, joint pain, fatigue, and complications of sacroiliitis, peripheral arthritis, spondylitis, osteomyelitis, and bursitis.

The most effective control measure to reduce animal brucellosis is largely based on vaccination of animals in endemic areas. However, much research in animal vaccination has been focused on development of live attenuated vaccines including *Brucella abortus* S19, *Brucella abortus* RB51, and *Brucella melitensis* Rev.1. *Brucella abortus* S19 is often considered a gold standard for vaccine development. Traditionally, live attenuated vaccines have a much broader use and efficacy than inactivated vaccine formulations. During the past few years, there have been an increasing number of studies on alternative approaches for development of more potent and safe vaccine against brucellosis including recombinant subunit vaccines using surface or intracellular proteins of *Brucella* spp, DNA vaccine, and a live vector vaccine.

The current text is dealing with field of brucella vaccinology in bovines including different types of brucella vaccines and its implantation in brucella control program.

Brucella Vaccines:

An ideal vaccine against brucellosis should possess the following characteristics: (i) be live and able to provide a strong type 1 T helper immune response (Th1); (ii) do not induce antibodies that interfere with the serological tests employed in the diagnosis of infected cattle, regardless of route, dose of administration, age or sex of the animals; (iii) be attenuated and do not cause disease or persistent infection in immunized animals nor

be pathogenic for humans; (iv) be able to induce a strong and long-lasting protection against systemic and uterine infection, besides preventing abortion, even in pregnant animals inoculated with a single dose; (v) do not lead to seroconversion on revaccination; (vi) be stable and do not revert virulence in vivo nor in vitro; and (vii) be inexpensive, easy to produce and to administer.

Measuring Efficacy and safety of vaccine:

Vaccines against brucellosis have been evaluated with respect to their potency by three different approaches: (i) testing in laboratory animals or (ii) testing in natural hosts experimentally challenged and (iii) testing under natural conditions. Test in natural hosts shows more significant response that measures the efficacy of *B. abortus* vaccines. Safety of vaccine is measured by ability of the vaccine in preventing abortion when most susceptible animals (mid gestation) is received a known infectious dose of virulent *B. abortus* strain. Field efficacy of vaccine can be influenced by other factors, such as nutrition, environmental stress, age at vaccination, vaccination management or immunological status..

Quality of the vaccine:

Assessing the quality of live *Brucella* vaccines is usually based on physicochemical and microbiological parameters. Different in vitro tests are performed to determine purity, dissociation and pH, humidity and count of viable bacteria. Genetic stability has also been proposed as an additional criterion in assessing of the quality of *Brucella* spp. vaccines. Live attenuated *B. abortus* strains have demonstrated the best results in the prevention of bovine brucellosis. Besides, many other *B. abortus* vaccine candidates have been developed, including DNA, subunit, recombinant *B. abortus* and recombinant vector vaccines. All of them are evaluated principally

in mouse model and the majority of these new vaccines, have not been tested in cattle.

Live Attenuated *B. abortus* vaccines

Live attenuated vaccines against *B. abortus* for immunization in cattle are RB51, S19 and 45/20. These strains are derived spontaneously from primary strains. Live attenuated vaccines provide the desirable protection over other types of *Brucella* strains because they have all the immunogenic components of replication and cell invasion and can induce diverse immunity in the host. In addition, they can prevent abortion and transmission of brucellosis, but may cause abortion in pregnant animals and are virulent for humans.

***Brucella abortus* S19 vaccine**

Brucella abortus S19 is the first *B. abortus* vaccine to be used extensively for bovine brucellosis control. It was isolated in 1923 from milk of a Jersey cow by Dr John Buck. This virulent culture was accidentally left out at room temperature for one year showing lower virulence than wild strain. Later on S19 showed to be highly successful in immunization of calves against brucellosis. In calves, S19 vaccination can be performed with full dose [$2.5-12 \times 10^{10}$ colony forming units (CFU)], original dosage, or with reduced dosage ($3-10 \times 10^9$ CFU) to minimize residual antibody titers.

S19 adult vaccination is not practised because vaccine strain may produce persistent vaccinal antibody and may cause abortion in pregnant animals. It also interfere diagnostic test by inducing anti LPS antibodies. Nonetheless, some cattle remain chronically infected and may abort and excrete the vaccine strain in the milk. In males, calf hood S19 vaccination usually results in persistent antibody titers, testicular infection, and hence infertility. Furthermore, the vaccination of infected animals with S19 does not cure nor alter the normal course of the disease.

Duration of immunity induced by S19 in cattle vaccinated as calves has proven to be quite long, reaching almost the entire productive lifespan of the animal. Moreover, revaccination experiments with S19 and killed B. abortus vaccines demonstrated no apparent benefit in cattle-challenge experiments compared with just S19-calfhood immunization. S19 vaccine strain trigger strong Th1 immune response with production of IFN- γ and high levels of antigen-specific CD4+ and granzyme B-secreting CD8+ T-cell responses.

Brucellosis control program using B abortus calfhood-S19 vaccination

Calfhood vaccine is produced from B abortus -S19 strain, which is low virulence, smooth Brucella strain expressing O side chain (perosmine residue) on its lipopolysaccharides (LPS). This attenuated strain multiplies within the body of the animal for a shorter period of time than the virulent field strain from which it was derived. B. abortus S19 lacks erythritol catabolic gene rendering it sensitive to erythritol. The strain differs from B. abortus biovar-1, by its inability to grow in the presence of dye thionine blue, penicillin and erythritol.

Calfhood vaccination is practised in females of 4-8 months age. Maternal antibody protects calfs upto 4 months of age. Therefore calfhood vaccination is less effective in calves carrying maternal antibodies.

To reduce the human health hazard and economic losses to livestock farmers, brucellosis control program has been extended in 12th five year plan by GOI with

calfhood vaccination of all female calves of 4-8 months age.

The major advantages of calfhood vaccination includes:

- (I) It prevents abortion in herds,
- (ii) No repeat or booster vaccination is required
- (iii) It prevents spread and builds up herd immunity in 3-5 years of period

The disadvantages are:

- (I) Restriction on age of vaccination, due to the interference in the brucellosis diagnosis, is the main disadvantage of vaccination with S19.
- (ii) Vaccination in pregnant animals result in abortion
- (iii) Calfhood vaccine is excreted in semen hence male animal cannot be vaccinated
- (iv) Accidental injection, inoculationconjunctival splashing while vaccine volumes in syringe or contact of vaccine on abraded skin result in human infection

Being pathogenic to man, the utilization of S19 vaccine requires safety training of the personal involved and the use of personal protection equipment as gloves, long sleeve coats, protection glasses, and masks

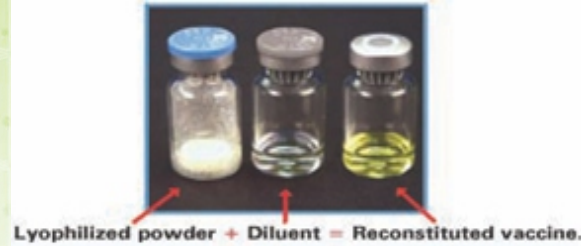
Source of Calfhood vaccine:

- (I) Bruvax (Indian Immunologicals Limited, Hyderabad) :Live freeze dried S-19 for female cattle and buffalo calves of age 4 to 8 months given 2.0ml subcutaneously.
- (ii) Brucella vaccine living (Intervet India Pvt. Ltd., Pune) for cattle and buffalo calves of age 4 to 8 months given 2.0ml subcutaneously.

Vaccination Procedure



Bruvax: Live freeze dried S-19 vaccine



Reconstitute lyophilized vaccine with diluent



Inject 2.0 ml S/C
4-8 months age
calves

Reconstituted Vaccine for injection



Dispose used syringe and empty vaccine vials in biohazard bags

Rb51 Vaccine

B. abortus strain RB51 is a rough rifampicin-resistant strain, which exhibited a lack of expression of the LPS O-side chains (OPS). RB51 vaccine strain was developed in 1982 by Prof. Gerhard Schurig's group and is derived from a virulent smooth *B. abortus* biovar 1 strain 2308. This is a natural mutant derived by a serial passages on media containing sub-inhibitory concentrations of rifampicin or penicillin and by selecting single colonies with rough morphology.

Calves vaccinated with RB51 at three, five

and seven months of age are protected against infection and abortion. Vaccination with S19 is slightly (not significant) more efficacious than RB51. After vaccination, RB51 is usually cleared from calf superficial cervical lymph node within 6 to 10 weeks. RB51 is considered more attenuated than S19, based on results of clearance and histologic examination of infected tissues of vaccinated animals.

In general, the recommended dosage for RB51 calfhood vaccination is $1.0 - 3.4 \times 10^{10}$ CFU. Protection against *B. abortus* infection is similar

through the suggested dosage, Reduced dosage (1×10^9 CFU), generally recommended for adult animals, also protects against infection and abortion caused by virulent 2308. It is not completely safe for pregnant cow, mainly when full dose is administrated.

Because of the rough phenotype, RB51 does not induce the production of anti-OPS antibodies in immunized animals, overcoming the serologic problems observed after S19 vaccination. Consequently, RB51 vaccinated cattle can be easily and accurately differentiated from naturally infected animals (DIVA strategy), allowing the effective use of the test-and-slaughter and vaccination policies simultaneously.

45/20 vaccine

This vaccine is prepared with heat-killed *B. abortus* biovar 1 strain 45/20 combined with oil adjuvant. The 45/20 is a rough *B. abortus*, derived of smooth strain 45/0 after 20 passages through guinea pigs. This vaccine is used for *B. abortus* control replacing S19, in order to eliminate the problems related to the induction of antibodies interfering in the routine diagnosis of infection.

New generation Brucella vaccine

Live attenuated vaccines are predominately used for bovine brucellosis control program which are produced from spontaneously attenuated or randomly selected strains. Recently, advance genomics, proteomics, recombinant DNA technology and even in vaccinology, allowed the exploration of other tools for the development of safer vaccines that nullify drawbacks observed in classical vaccines. therefore, several studies have been performed particularly in mice to test the efficacy or assess the immunological responses of the *B. abortus* genetically engineered vaccines i.e. recombinant genes, proteins, vectors and modified *B. abortus* strains. With a few exceptions the majority of these recombinant vaccines, have not been tested or did not protect cattle.

However, these vaccines have several advantages over the classic live attenuated vaccines, which include, high safety without residual virulence,

and possible use in humans and pregnant animals.

DNA vaccines

DNA vaccines offer the possibility of inducing both cellular and humoral responses with prolonged immunity. They have better stability and do not require refrigeration under storage. DNA vaccines encoding ribosomal L7/L12, lumazine synthase (BLS), P39 (a putative periplasmic binding protein), Omp16 (outer membrane protein) and BAB1_0278 genes have demonstrated to confer protection against *B. abortus* challenge in mice. Moreover, Cu/Zn superoxide dismutase (Cu-Zn SOD) DNA vaccine induced a protection level similar to the one induced by RB51. All these genes also proved capable of eliciting a desirable cellular immune response in mice.

Combined DNA vaccines have also demonstrated their ability to protect better against challenge. This combined DNA vaccine also elicited significantly higher cytotoxic response (granzyme B-producing CD8+ T cells) compared to S19-vaccinated mice. DNA vaccine candidates have shown very promising results in mice, but the major drawback of DNA vaccines in large animals include its higher cost and it requires multiple booster vaccinations to be effective.

Subunit vaccines

Different fragments of *Brucella* including recombinant peptide, protein, DNA, lipopolysaccharide (LPS), and outer membrane vesicles (OMVs) have been evaluated as subunit vaccines against *B. abortus*. Although they are attractive alternatives to the classic live attenuated vaccines, they do have certain shortcomings. These include low protection efficacy and the need for adjuvant and booster shots. Powerful T-cell antigens can be used to enhance protection levels of subunit vaccines which in turn induce Th1 immune response against brucellosis.

Brucella protein subunit vaccines are OMP16, OMP19, liposomized protein L7/L12, OMP25, p39 (a putative periplasmic binding protein), and AsnC. These promote Th1 type

immunity and impart protection levels that are comparable to the commercial S19 live vaccine. In contrast, dihydrolipoamide succinyl-transferase (rE2o) and cysteine synthase A (rCysK) subunit vaccines elicited Th2 type immunity, with relatively low levels of protection. Flagellar proteins such as FliN, FlgJ have been evaluated as subunit vaccine antigen candidate for their ability to induce humoral and cell mediated immune response..

Many of the same antigens tested as DNA vaccines have also been evaluated as potential antigens for subunit vaccines which include L7/L12 ribosomal protein; P39; BLS; Omp16; Cu/Zn SOD etc.

However, the potential use of *B. abortus* subunit vaccines under field conditions is very limited. The requirement of multiple boosters, adjuvants and combination of several antigens makes it economically unsuitable for cattle.

Vector vaccines

Genes encoding immunodominant *B. abortus* antigens can be introduced into attenuated viruses (like Vaccinia virus) or bacteria (like *Escherichia coli*) that serve as vector vaccines. Vector based vaccines are live and replicative in host cell and induce cell mediated immunity. , *Escherichia coli*, *O. anthropi* (plus unmethylated CpG motifs) and *L. lactis* expressing Cu/Zn SOD antigen of *B. abortus* were able to elicit a Th1 immune response and to protect mice following challenge with virulent *B. abortus*. The viral or bacterial vector based vaccines are superior alternative to DNA and subunit vaccines as it closely mimics the natural infection. However, it requires multiple doses and adjuvant for optimum efficacy.

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Other *B. abortus* potential vaccines

Vaccines based on outer membrane vesicles (OMVs) have been exploited as an acellular alternative to live vaccines. *B. abortus* OMVs are mainly composed for outer membrane proteins (Omps) and have been associated with modulation of host immune response. A *Brucella melitensis* vaccine based on OMVs has been tested and showed promising results in BALB/c mice . Therefore OMV vaccine against *B. abortus* has a great potential to be considered as part of the continuous efforts to reach safer and more effective *B. abortus* vaccine.

Conclusions

As per OIE guidelines, brucellosis control and eradication program constitutes of three successive phases including (i) mass vaccination, (ii) combination of vaccination and culling of seropositive animals, and (iii) test and slaughter. Vaccination associated with continuous elimination of infected animals is the determinant strategy for brucellosis control program in our country. Some effective vaccines including S19 and RB51 are approved *B. abortus* vaccine strains more widely and successfully used to prevent bovine brucellosis worldwide. However, due to some side effects shown by these current vaccines there is an ongoing extensive efforts focused on the development of new and better vaccines. Engineered vaccines have the potential to be the future of the bovine and human brucellosis control. But many studies are still needed to develop a better vaccine than the current vaccines in terms of safety, efficacy and other desirable characteristics.

CANINE BABESIOSIS: AN OVERVIEW

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Introduction :

Canine babesiosis is a life threatening disease throughout the globe, caused by intraerythrocytic apicomplexan parasites of the genus *Babesia*. Out of the two types of *Babesia* encountered in dogs, the large form, hitherto known as *Babesia canis*, has now been assigned to three sub-species, viz., *B. canis canis* (prevalent in Europe, moderately pathogenic), *B. canis rossi* (restricted to sub-Saharan Africa and Southern Africa, highly pathogenic) and *B. canis vogeli* (widely distributed in tropical and sub-tropical countries, mildly pathogenic). The variations on geographical distribution, vector specificity, cross-immunity and serology prompted some authors to elevate these three sub-species to species status, viz., *B. canis*, *B. rossi* and *B. vogeli* (Irwin, 2009; Schoeman, 2009). *B. gibsoni* is a small pleomorphic organism measuring between 1 to 2.5µm and appears most commonly as ring form or rarely large ovoid to elongate forms stretching across the erythrocytes. It is found in almost all parts of Asia, Europe, Africa, America and Australia (Conrad et al., 1991; Casapulla et al., 1998).

Genotyping of Babesia

Traditionally, all small canine piroplasms are recognized as *B. gibsoni* and it is assumed that no other small *Babesia* species infect dogs. This hypothesis was proved wrong by characterization of small piroplasms of dogs from Spain and California (Kjemtrup et al., 2000; Zahler et al., 2000a). Genetic characterization and phylogenetic analysis of the 18S rRNA gene that included canine small piroplasms from Asia, the Midwestern United States, California, Africa and Spain conclusively proved that there are three genotypically distinct small *Babesia* of canines (Kjemtrup et al., 2000); *B. gibsoni sensu stricto* from Asia and the Midwestern United States

(Zahler et al., 2000b), *B. microti*-type from Spain, now named as *Theileria annae* (Zahler et al., 2000a; Garcia, 2006) and the Californian isolate, now known as *B. conradae* (Kjemtrup and Conrad, 2006). Some authors divided the small canine piroplasms into three distinct clades. *B. gibsoni* has been categorized under *Babesia sensu stricto* clade, *B. conradae* has been kept in Western clade and the ancestral *B. microti*-type isolate has been assigned to the *Babesia microti* clade. Few other small piroplasms, viz. *Theileria equi*, *T. annulata* and an unnamed *Theileria* sp. were also reported in canines from Spain and South Africa (Matjila et al., 2008). As the clinical picture, choice of drug for treatment as well as epidemiology and control strategies of the disease varies depending upon the genotype of the parasite in question, it is imperative to ascertain the species, sub-species/genotype involved in canine babesiosis. Fortunately, there is no information available on genotypes of *B. gibsoni* prevalent in India (Mandal et al., 2014).

Clinical pathology:

B. gibsoni may cause hyperacute, acute, and more commonly, chronic infection. Hyperacute infection is rare, mainly occurring in pups leading to rapid death. Acute infections are typically associated with remittent fever, progressive anaemia, lethargy, thrombocytopenia, haemoglobinuria, marked splenomegaly and hepatomegaly (Goo et al., 2008). Convulsions and muscular twitching has also been reported (Varshney et al., 1997). Chronic infections are more common and may be completely asymptomatic or may be characterized by intermittent fever, lethargy and weight loss. Chronically infected dogs remain as carriers without any overt clinical sign (Conrad et al., 1991). Concurrent infection of *B. gibsoni* and other blood parasites, viz., *B. canis*, Hepatozoon

canis, Ehrlichia canis has also been reported frequently (Dantas-Torres and Figueredo, 2006). Infection with B. gibsoni generally results in more severe clinical manifestation than infection with B. canis and may cause multiple organ dysfunctions.

Transmission:

In nature, B. gibsoni is transmitted through tick-bite. The potential vectors for Californian isolate of B. gibsoni (B. conradae) are Rhipicephalus sanguineus and Dermacentor variabilis in which transovarian or transtadial transmission occur (Kjemtrup and Conrad, 2006). Ixodes hexagonus is the main vector for transmission of Theileria annae in Spain (Camacho et al., 2003). In India, Rhipicephalus sanguineus, the brown dog tick, is the sole vector known to transmit B. gibsoni and B. canis. Apart from transmission through ticks, there are evidences that B. gibsoni infection may also be transmitted via dog bites (blood-to-blood contact during dog fighting) and blood transfusion as well as via the transplacental route to the developing foetus (Fukumoto et al., 2005).

Diagnosis:

Microscopical & Serological method

Chronic form of Babesia gibsoni with very low parasitaemia is more common than acute or hyperacute forms. Hence due care should be taken for diagnosis of chronic infections (Conrad et al., 1991). Diagnosis of canine babesiosis is classically made by light microscopic demonstration of intra-erythrocytic parasites in Romanowsky stained blood smears. But in subclinical or latent infection, this may be fastidious due to its poor sensitivity (Goo et al., 2008). Serological tests like immunofluorescent antibody test (IFAT) and enzyme-linked immunosorbent assay (ELISA) with whole parasites or native antigen have been proved to be useful for the diagnosis of chronic infection and field surveys but they have limited application due to poor quality of antigens and cross reactivity (Aboge et al., 2007b). Of late, recombinant proteins based ELISA and immunochromatographic tests (ICT) have been

attempted with promising results (Fukumoto et al., 2001a; Zhou et al., 2006a,b; Aboge et al., 2007a; Jia et al., 2007; Goo et al., 2009a; Goo et al., 2012b). Recombinant antigens are more defined and thus, the chances of cross-reactivity with other parasites is unlikely.

Molecular diagnosis:

Molecular techniques have also been used successfully for diagnosis of B. gibsoni infection. Polymerase chain reaction (PCR) has been used with good sensitivity and specificity (Ano et al., 2001; Fukumoto et al., 2001b) and was able to detect early or carrier infections (Birkenheuer et al., 2003). PCR derived tools such as PCR-restriction fragment length polymorphism (RFLP) and nested PCR also offer an effective and rapid means of discrimination between canine Babesia species and sub-species (Lemos et al., 2012). Though PCR is most commonly used for diagnosis of B. gibsoni infection, recent introduction of loop-mediated isothermal amplification (LAMP) has resulted in development of another rapid, simple, sensitive and specific diagnostic method, which is less time consuming in comparison to PCR (Ikadai et al., 2004).

Treatment:

Treatment of canine microbabesiosis (caused by B. gibsoni) is difficult in comparison to macrobabesiosis (caused by B. canis). In practice, diminazene aceturate and imidocarb dipropionate are extensively used for the treatment of canine babesiosis but relapses are quite common in dogs infected with B. gibsoni (Kjemtrup and Conrad, 2006). A combination therapy with autovaquone and azithromycin has been claimed to clear B. gibsoni infection and is presently the treatment of choice. However, the cost of this therapy is prohibitive for its use in veterinary practice (Lin et al., 2012).

Conclusion:

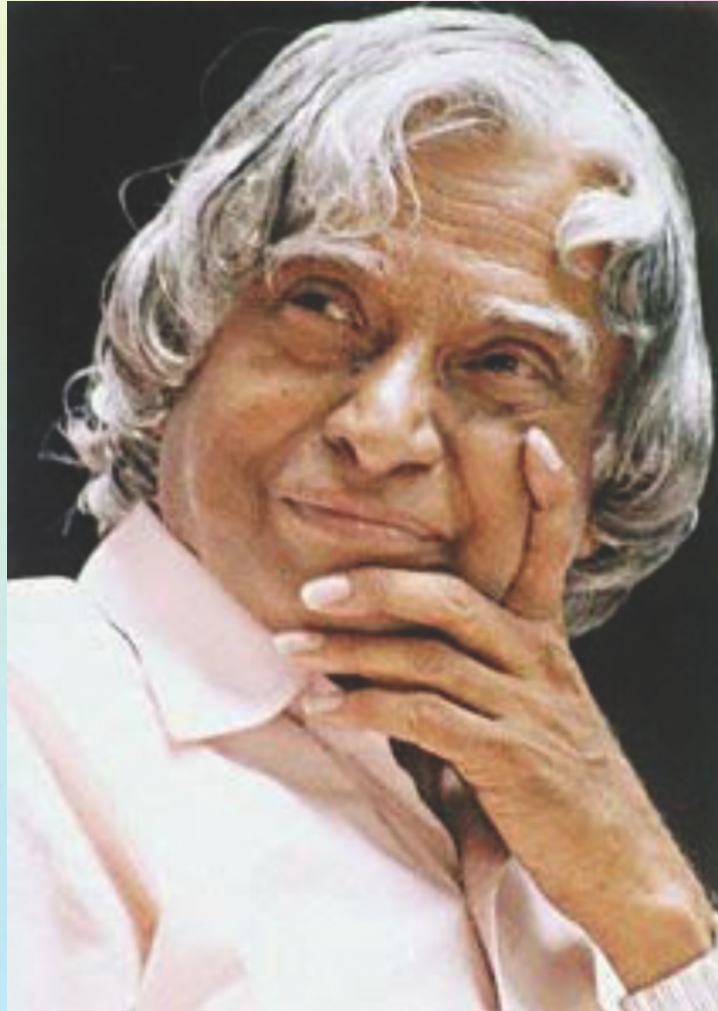
The prevalence of tick-borne haemoprotozoan diseases in India is quite high because of favourable climatic conditions characterized by high temperature and humidity, which favour rapid multiplication of the tick vector. However,

the epidemiology and clinical importance of *B. gibsoni* infections in India are not well understood. In fact the occurrence of *B. gibsoni* infection in dogs has been sporadically reported on the basis of microscopic examination of blood smears. In a recent survey, India's dog population was estimated at 25 million and 17% of the households were reported to own a pet/domesticated dog. Moreover, increased use

of dogs in national security warrants for better diagnosis and management of canine health problems. The available literature, although limited, suggests that canine babesiosis, caused by both *B. canis vogeli* and *B. gibsoni* is endemic throughout India. There is an urgent need to develop a rapid and accurate diagnostic tool based on the knowledge of prevalent species and their genotypes.

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