Reproductive Management of Dairy Animals

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EDITION

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Reproductive Management of Dairy Animals

Programme Coordination
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This e-book is a compilation of resource text obtained from various subject experts of SVU-GADVASU & MANAGE, Hyderabad, Telangana on “Reproductive Management of Dairy Animals”. This e-book is designed to educate extension workers, students, and research scholars, academicians related to veterinary science and animal husbandry about “Reproductive Management of Dairy Animals”. Neither the publisher nor the contributors, authors and editors assume any liability for any damage or injury to persons or property from any use of methods, instructions, or ideas contained in the e-book.

Published for Dr.P.Chandra Shekara, Director General, National Institute of Agricultural Extension Management (MANAGE), Hyderabad, India by Dr. Srinivasacharyulu Attaluri, Program Officer, MANAGE and printed at MANAGE, Hyderabad as e-publication.

MESSAGE

National Institute of Agricultural Extension Management (MANAGE), Hyderabad is an autonomous organization under the Ministry of Agriculture & Farmers Welfare, Government of India. The policies of liberalization and globalization of the economy and the level of agricultural technology becoming more sophisticated and complex, calls for major initiatives towards reorientation and modernization of the agricultural extension system. Effective ways of managing the extension system needed to be evolved and extension organizations enabled to transform the existing set up through professional guidance and training of critical manpower. MANAGE is the response to this imperative need. Agricultural extension to be effective, demands sound technological knowledge to the extension functionaries and therefore MANAGE has focused on training program on technological aspect in collaboration with ICAR institutions and state agriculture/veterinary universities, having expertise and facilities to organize technical training program for extension functionaries of state department.

In India, dairy industry comprises a major part of the agricultural economy where cattle and buffaloes are mainly reared by small and marginal farmers. Impaired reproductive efficiency of dairy animals is a major concern in improving the milk production. Infertility among male as well as female dairy animals is a problem routinely encountered by the field veterinarians. It is important to have knowledge about the recent diagnostics and therapeutic measures of major reproductive disorders of dairy animals.

It is a pleasure to note that, SVU- Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana, Punjab and MANAGE, Hyderabad, Telangana is organizing a collaborative training program on “Reproductive Management of Dairy Animals” from 7-9 July, 2021 and coming up with a joint publication as e-book on “Reproductive Management of Dairy Animals” as immediate outcome of the training program.

I wish the program be very purposeful and meaningful to the participants and also the e-book will be useful for stakeholders across the country. I extend my best wishes for success of the program and also I wish SVU- GADVASU, Ludhiana, Punjab many more glorious years in service of Indian agriculture and allied sector ultimately benefitting the farmers. I would like to compliment the efforts of Dr. Shahaji Phand, Center Head-EAAS, MANAGE, Hyderabad and Dr. Parkash Singh Brar, Director Extension Education, SVU-GADVASU, Ludhiana for this valuable publication.

Dr. P. Chandra Shekara
Director General, MANAGE

MESSAGE

Livestock in the developing world endure distinctive challenges from their environments, which are generally harsher and less managed than those experienced by livestock in developed countries. In these circumstances reproduction of dairy animals is mostly affected. Optimum reproduction processes are prerequisite to obtain satisfactory production from dairy animals. Several reproductive disorders impede the process leading to huge economic loses to dairy industry.

It is a matter of immense pleasure that Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Sciences, in collaboration with Directorate of Extension Education, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana and MANAGE, Hyderabad is organizing an online training programme based on "Reproductive Management of Dairy Animals" from 07-09 July, 2021 for the Extension officials of state/central animal husbandry departments, veterinarians, faculty of SAUs/SVUs/KVKs/ICAR institutes, etc.

The e-book generated out of this online training is meticulously designed to expose the trainees to various aspects of advanced diagnostic and therapeutic approaches towards different reproductive disorders of dairy animals. I hope that the participants from different parts of the country would be enormously benefitted.

The Department of Veterinary Gynaecology and Obstetrics, GADVASU is designated as Centre of Advanced Faculty Training by Indian Council of Agricultural Research, New Delhi for its outstanding contribution in the field of Animal Reproduction.

I would like to take this opportunity to congratulate Department of Veterinary Gynaecology and Obstetrics, GADVASU and MANAGE for their fruitful collaboration towards benefits for the farmer community. I also congratulate the course coordinator Dr. Mrigank Honparkhe, Principal Scientist-cum-Head, and course co-coordinators for their untiring work and high level of enthusiasm.

Dr. Parkash Singh Brar
Director Extension Education,
GADVASU

PREFACE

This e-book is an outcome of collaborative online training program on "Reproductive Management of Dairy Animals" is conducted with the intention of providing knowledge and hands-on experience on latest techniques to diagnose and treat various reproductive disorders of cattle and buffaloes to veterinary practitioners. This e-book is intended to give the field veterinarians, a detailed understanding of various etiologies, diagnostics and treatment of different gynaecological, obstetrical and andrological disorders of dairy animals.

Reproduction is a vital component in the animal husbandry practices which is mostly affected during any adverse condition. In the field conditions the clinical cases pertaining to infertility, dystocia, conception failure etc. are most commonly encountered by the veterinarians. The knowledge about the recent advances in the diagnostic and therapeutic management of reproductive disorders in dairy animals is a prerequisite for improving the reproductive efficiency followed by milk production and thus helping in doubling the dairy farmer's income. This book is incorporated with details about fixed timed artificial insemination protocols, therapeutics of anestrus and repeat breeding syndrome, obstetrical interventions like handling of uterine torsion, incomplete cervical dilatation, tools like fetotomy, caesarean section, various peri-parturient problems like retention of fetal membranes in bovines. We have also included basics of reproductive ultrasonography and its use to diagnose pathological conditions in male and female animals. Various strategies to diagnose and treat uterine infections especially uterine cytobrush technique; a novel approach to diagnose endometritis has also been discussed in e-book.

There is inclusion of various aspects of male reproductive management like breeding soundness evaluation, semen evaluation and artificial insemination.

The financial assistance provided by National Institute of Agricultural Extension Management (MANAGE), Hyderabad for conducting this training and generating e-book is duly acknowledged.

The valuable suggestions for future improvements are always welcome.

July, 2021

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CONTENTS

Fixed Time AI Protocols: A Tool for Effective Reproductive Management of Dairy Animals

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Estrus synchronization is one of the most important and widely applicable reproductive biotechnologies available for cattle. The major factor limiting optimum reproductive performance on many cattle farms is failure to detect cows in heat in a timely and accurate manner. Poor estrus detection results in excessive number of days open which causes long calving intervals and economic losses to the farmers. A study conducted to evaluate efficiency of visual estrus detection showed that only 56% of cows observed twice a day for 30 minutes could be detected in standing estrus as compared to 95% of cows detected following 24 hours a day visual monitoring (Downing et al., 1998). This shows that estrus detection is time consuming and labour intensive process.

Estrus synchronizing is an effective way to minimize the time and labour required to detect standing estrus for artificial insemination. In addition, some estrus synchronization protocols (progestin-based protocols) can induce a proportion of anestrous cows to begin estrous cycles providing more chances for cows to conceive during a defined breeding season.

Estrus synchronization

Estrus synchronization is the manipulation of the reproductive process i.e. estrous cycle so that a group of females exhibit standing estrus and can be bred with normal fertility during a short, predefined time interval. This control facilitates breeding in two important ways i.e. it reduces and in some cases eliminates the labour of detecting estrus (heat), and it allows the farmers to schedule the breeding.

Basic approach for estrus synchronization

Basic approach is to control the timing of the onset of estrus by controlling the length of the estrous cycle. Two approaches are used for controlling estrous cycle length -

1. *Shortening of Luteal phase* – by administration of Prostaglandin ($PGF_2\alpha$) to regress the corpus luteum (CL) before the time of natural luteolysis.
2. *Prolongation of Luteal phase* – by administration of Progesterone or more commonly synthetic progestins to temporarily prolong the luteal phase or delay onset of estrus.

Estrus Synchronization Protocols

A variety of estrus synchronization protocols are available which involves use of hormones like Prostaglandins, GnRH, Estradiol esters, progesterone or progestagin devices. The PGF protocols does not facilitate fixed time insemination whereas, most of the GnRH based and estradiol based protocols facilitate fixed time insemination and are widely used.

Prostaglandins

Prostaglandin ($PGF_2\alpha$) is a naturally occurring hormone. During the normal estrous cycle of a non-pregnant animal, $PGF_2\alpha$ is released from the uterus 16 to 18 days after the animal was in heat. This release of $PGF_2\alpha$ functions to destroy the corpus luteum (CL). The CL is a structure in the ovary that produces the hormone progesterone and prevents the animal from returning to estrus. The release of $PGF_2\alpha$ from the uterus is the triggering mechanism that results in the animal returning to estrus every 21 days. Commercially available $PGF_2\alpha$ (Lutalyse, Estrumate, Clostenol etc.) gives the herd owner the ability to simultaneously remove the CL from all cycling animals at a predetermined time that is convenient for heat detection and breeding.

The major limitation of $PGF_2\alpha$ is that it is not effective on animals that do not possess a CL. This includes animals within 6 to 7 days of a previous heat, prepubertal

heifers and postpartum anestrous cows. Despite these limitations, prostaglandins are the simplest method to synchronize estrus in cattle.

Single Shot PGF or 6-Day Heat Detection Plus PGF

A lower cost alternative is to breed animals to natural heats for 6 days and then inject the unbred animals with $PGF_2\alpha$ and breed over the next 5 to 7 days. This system allows all cycling animals to be bred during a two week period and requires less PGF injections/head. Although this system is conservative in terms of hormone usage, it is probably one of the more labor intensive synchronization options. If <20% of the animals have been inseminated following 6 days of heat detection, there may be a cyclicity problem. Don't waste time and money trying to synchronize a herd of cows that are not cycling. Instead, evaluate the body condition score, herd health and nutrition level of the herd.

Two-Shot PGF Protocol

The most common method of synchronization with $PGF_2\alpha$ is to inject all animals and breed those that come into heat over the next 5 to 7 days. Animals not detected is estrus after the first injection are re-injected 14 days later and bred over the next 5 to 7 day period. Animals detected in standing heat should be inseminated 8-12 hours later. If labor availability is a limitation, all heat detection and breeding can be delayed until after the second PGF injection. This allows the producer to breed a high percentage of the herd during a single 5-7 day period, but requires two doses of PGF/head versus 1.3 to 1.5 doses/head if animals are bred after each injection. Overall estrus response rates may be slightly reduced (~5%) when animals are bred only after the second injection as some animals that responded to the first injection may not respond again to the second.

Although recommendations were to inject $PGF_2\alpha$ at 11-day intervals, from a scheduling consideration, the 14-day interval is much easier to implement. The second injection is always 2 weeks down on the calendar from the first and all activities (injections, heat detection, breeding) are conducted on the same days of the week from one week to the next. Animals that respond to the first injection, but are not detected in

estrus, will be between day 7 and 9 of the cycle at the next injection using the 11-day interval. These "early" CLs typically do not respond to PGF as well as older more mature ones. Using a 14-day interval, a missed heat from the first injection will be on days 10 to 12 of the cycle at the second injection. This 3-day difference significantly improves the probability of the animal responding again.

PGF Limitations

i. *Fixed-time AI* - Fixed-time insemination after single or double injections of PGF alone seldom yields acceptable results and in general, is not recommended.
ii. *Suckled cows* - A major limitation of PGF is that it only works in cycling animals. Therefore, PGF-based protocols work very well in properly managed beef or dairy heifers and in many dairy herd systematic breeding programs. However, even in the best managed herds, some of the suckled cows may still be anestrus at the beginning of the breeding season. In such situations, use of PGF in combination with GnRH and/or a progestin source are much more effective options.
iii. Estrus and ovulation is highly variable due to differences between cows in the stage of follicular development at the time of PGF injection.

Lot of variation has been reported in onset of estrus following administration of $PGF_2\alpha$ which is mainly attributed to the ovarian follicular status. Therefore, a PGF protocol does not facilitate fixed time insemination of cows. To facilitate fixed timed insemination, GnRH and Estradiol based protocols have been developed which are based on exogenous control of follicular wave emergence. Therefore, it's important to understand the role of ovarian follicular waves in estrus synchronization.

Follicular Waves

Follicles are blister-like structures that grow on the ovaries. Each follicle contains an unfertilized egg that will be released to the oviduct if the follicle ovulates. The follicular growth occurs in waves throughout the estrous cycle. Each wave is characterized by rapid growth of numerous small follicles. From this wave of follicles,

one follicle is allowed to grow to a much larger size than the others (12 to 15 mm). This large follicle is called the dominant follicle because it has the ability to regulate and restrict the growth of other smaller follicles. A few days after reaching maximum size, the dominant follicle begins to regress. As the dominant follicle regresses, it looses the ability to restrict the growth of other follicles. Thus, a new follicular wave is initiated coinciding with the regression of the previous dominant follicle. From the new follicular wave, another dominant follicle will be selected. Most cows will have two or three follicular waves during an 18 to 24 day cycle.

Follicular waves and PGF

Any dominant follicle has the capacity to ovulate provided the inhibitory effects of progesterone can be removed at an opportune time. Prostaglandins serve this function by destroying the CL, however, PGF has no direct effect on the normal pattern of follicular waves. Thus, the stage of follicular development at the time of PGF injection will affect the interval from injection to standing estrus. Animals injected when the dominant follicle is in the growing phase will display estrus within 2 to 3 days, whereas animals with aged or regressing dominant follicles (C) may require 4 to 6 days before a new follicle can be recruited for ovulation.

Follicular waves and GnRH

An injection of GnRH causes a release of Luteinizing Hormone (LH) from the pituitary gland in the brain. This LH "surge" results in ovulation or luteinization of most large dominant follicles. A new "synchronized" follicular wave is initiated in these animals 2 to 3 days later. Because GnRH stimulates development of luteal tissue in place of the dominant follicle, a higher percentage of cows will possess sufficient luteal tissue to respond to PGF 7 days later. Injecting cows with PGF 7 days after a GnRH injection synchronizes luteal regression in animals with previously synchronized follicular development. The result is a higher estrus response rate and much better synchrony of estrus as compared to PGF alone.

Although GnRH synchronizes follicular development in most cows, some cows do not respond to the first GnRH injection. If the GnRH injection fails to luteinize a follicle in animals that were due to show heat naturally around the time of the PGF injection, the treatment fails to prevent those animals from displaying estrus as they normally would. Research in both beef and dairy cows has consistently revealed that 5 to 10% of cows treated with GnRH will display standing estrus 6 to 7 days later. These natural heats should be bred when detected and subsequent injections are not administered. GnRH-based synchronization protocols are not currently recommended in virgin heifers because they do not respond to GnRH injections as consistently as do mature cows.

GnRH-PGF Based Synchronization Protocols

Numerous new synchronization protocols currently recommended for cows use gonadotropin-releasing hormone (GnRH) in conjunction with PGF. Each GnRH-based protocol uses the same basic framework, which involves an injection of GnRH followed 7 days later with an injection of PGF. The way animals are subsequently handled for heat detection and breeding is where the protocols begin to vary. It is important to understand the concept of follicular waves in cattle to understand the benefits of GnRH-based synchronization protocols and how they work.

Select Synch

With the Select Synch System, cows are injected with GnRH and PGF 7 days apart. Heat detection begins 24-48 hours before the PGF injection and continues for the next 5-7 days. The PGF injection is excluded for cows detected in estrus on day 6 or 7. Animals are inseminated 8 to 12 hours after observed in standing estrus. Alternatively, heat detect and A.I. until 48 to 60 hours after PGF and then mass-AI the rest of the herd at 72 hours and give GnRH to those cows that have not exhibited estrus.

Major benefits of the Select Synch system are simplicity and tighter synchrony of estrus. Most animals will display standing estrus 2 to 4 days after the PGF injection. Overall, estrus response rates in well-managed beef herds average ~70 to 75% with no

adverse effect on conception rates (60 to 70%), resulting in synchronized pregnancy rates that average between 45 and 50%.

Select Synch followed by heat detection and 72 hour fixed time A.I. allows producers to maximize potential pregnancy rates while minimizing labor requirements for estrus detection (7,8). Heat detection is used to catch the early cows and to breed the majority of the herd (60 to 70%) to standing heats. Estrous detection can be terminated at 48 to 60 hours after PGF followed by mass-AI of the non-responders at 72 hours with GnRH. This option gives all cows an opportunity to conceive and, compared to strict fixed-time AI options such as Ovsynch and Cosynch, drug costs are reduced as only 30 to 40% of the herd will receive the second GnRH injection. Additionally, if less than 40 to 50% of the herd is detected in estrus by 72 hours, the mass mating can be aborted, saving drugs, money and semen that might otherwise be wasted on anestrous cows.

Select Synch resulted in more cows in standing estrus, equal or better conception rates and ultimately more cows pregnant during the synchronized breeding period. These benefits were particularly evident in the anestrous cows where estrous response rates were improved by 25% and conception rates (66%) were comparable to those of cycling cows. The Select Synch system more than doubled the percentage of anestrous cows that became pregnant during the synchronized breeding period.

Ovsynch

Ovsynch is a fixed-time AI synchronization protocol that has been developed, tested and used extensively in dairy cattle . The protocol builds on the basic GnRH-PGF format by adding a second GnRH injection 48 hours after the PGF injection. This second GnRH injection induces ovulation of the dominant follicle recruited after the first GnRH injection. All cows are mass inseminated without estrous detection at 8 to 18 hours after the second GnRH injection. Across large numbers of dairy cattle, pregnancy rates to Ovsynch generally average in the 30 to 40% range. Although these numbers may not appear impressive at first, it is important to understand them in terms of an applied reproductive management program.

Ovsynch pregnancy rates in dairy herds can be significantly improved if cows are set-up or "pre-synchronized" to be in the early luteal phase of the estrous cycle at the time of the first GnRH injection. This can be accomplished with 2 injections of PGF given at 14-day intervals with the last injection administered 12 to 14 days prior to starting Ovsynch.

Although Ovsynch allows for acceptable pregnancy rates with no heat detection, it does not eliminate the need for heat detection. Ovsynch treated animals should be observed closely for returns to estrus 18 to 24 days later. Additionally, natural heats can occur on any given day and as many as 20% of cows will display standing estrus between days 6 and 9 of the Ovsynch protocol. Conception rates in these animals will be compromised if bred strictly on a timed AI basis.

Cosynch

Although Ovsynch has proven to be a reliable timed AI program for beef cows as well, Ovsynch requires four trips through the working chute. Research at Colorado State University demonstrated that comparable pregnancy rates can be achieved with only animal handlings by breeding all cow coinsiding with the second GnRH injection. Thus, the name Cosynch. As with any fixed time AI protocol, results to Cosynch can be variable, but in general range from 40 to 50%. As with Ovsynch, pregnancy rates are maximized if the early heats are visually detected and bred using the AM/PM rule.

MGA - PGF System

The MGA-PGF system is a time tested, proven method for synchronizing estrus in beef and dairy heifers. Melengestrol Acetate (MGA) is a synthetic form of the naturally occurring hormone, progesterone. For best results, mix MGA with 3 to 5 lbs of a grain supplement and feed at a rate of 0.5 mg/ head/day for 14 days. Top dressing or mixing MGA in a TMR can work, but intake (and thus results) tends to be more variable. Within 3 to 5 days after MGA feeding, most heifers will display standing heat. DO NOT BREED at this heat as conception rates are reduced. Wait 17 to 19 days after the last day of MGA feeding, and inject all heifers with a single dose of PGF. For the next 5 to 7

days, inseminate animals 8 to 12 hours after detected estrus. Success of the MGA system depends on adequate bunk space and proper feeding rates so the appropriate dosage is consumed by each heifer on a daily basis.

With good heat detection of well-managed heifers at the proper age, weight and body condition, you can expect to achieve synchronized pregnancy rates of 50 to 70%.Because the synchrony of heats following the MGA-PGF protocol can be variable, pregnancy rates to single, fixed time inseminations are also variable. However, very acceptable pregnancy rates (45 to 55%) have been achieved to a single insemination at 72 hours or by double inseminating at 60 and 96 hours following the PGF injection.

1. Heat detect & AI for 3 to 5 days after removal
2. Fixed-time AI & GnRH at 50 to 60 hours after removal
3. Heat detect & AI 72 hours and fixed-time AI of non-responders with GnRH at 72 to 80 hours after removal

Eazi-Breedtm CIDR® Applications

The EAZI-BREED CIDR is a T-shaped vaginal insert that delivers the natural hormone progesterone during the 7-day treatment period. Cows and heifers receive Lutalyse (5 mL) on day 6 or 7after CIDR insertion with CIDR removal on day 7. Females are bred 8 to 12 hours after observed estrus for the next 3 to 5 days or at a single fixed time 48 to 64 hours after CIDR removal. Research indicates the extra animal handling to give Lutalyse on day 6 versus day 7 may reduce the average interval to estrus by about 12 hours with a slight improvement in synchrony of response, but will have no impact on the overall estrous response rate. Numerous research trials indicate an injection of GnRH at CIDR insertion may further improve synchronized reproductive performance, especially among anestrous cows. In other words, pregnancy rates of the many popular GnRH-PGF protocols such Ovsynch, CO-Synch and Select Synch are improved by inserting the CIDR at GnRH injection and removing the CIDR at the Lutalyse injection on day 7. Breeding cows and heifers to detected estrus for 72 hours after CIDR removal, followed by timed AI of non-responders with GnRH appears to minimize the herd to herd variation in pregnancy rates by breeding most cows to standing estrus with a minimum investment

in estrus detection labor, while the timed AI gives all females the opportunity to conceive.

MGA - Select

The MGA-Select system superimposes the MGA heifer protocol on the Select Synch protocol. Cows are fed MGA (0.5 mg/ head/day) for 14 days and treated with Select Synch starting 12 days after the last day of MGA feeding. As with Select Synch, cows are bred to observed heats for 72 to 80 hours after PGF and non responders are mass-mated with a concurrent injection of GnRH (Option 1). Alternatively, cows may be mass-mated with a concurrent GnRH injection at 72 to 80 hours after PGF. The MGA feeding helps to "jump start" cyclicity in many anestrous cows and presynchronizes cycling cows for optimum response to Select Synch. Numerous studies indicate the MGA Select system yields outstanding synchroinzed A.I. pregnancy rates ranging from 55 to 65% with both heat detection and fixed time A.I. breeding options. As with the heifer protocol, do not breed cows detected in estrus within 10 days of MGA feeding. ®MGA is a registered trademark of Pfizer Animal Health and is not approved for use in lactating dairy cows.

Management tips to maximize success

The major factor affecting the success of any estrus synchronization protocol is the percentage of animals cycling at the initiation of treatment. The single most important factor affecting cyclicity is nutrition. Feed cows to achieve a moderate or better body condition score by the time of calving and increase energy levels in rations to minimize the body condition loss. Perform all vaccinations at least three weeks ahead of the synchronization and breeding period to provide ample time for the immune system to respond and provide protection from the disease in question. First-calf heifers, late calving cows, difficult births, and retained placentas are all associated with reduced fertility. Group these "high risk" animals separately so maximum nutrition and veterinary care can be efficiently provided.

Suggested Readings

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Diagnostic Reproductive Ultrasonography in Dairy Animals

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Transrectal ultrasonography has been available for basic research since the early 1980s. This technology allows for the real-time visualization of internal structures such as ovaries, endometrium, and embryos or fetuses that are otherwise difficult to evaluate. Thus, the basic research discoveries that have been made possible through the use of transrectal ultrasonography can be translated to the producer to assist with making reproductive management decisions. Ultrasonography has numerous advantages over other imaging modalities. It is non-invasive, free from radiation hazards, provides instant diagnosis, and determines shape, size, location and internal consistency of a structure. Further, repetitive examinations can be done and it is well tolerated by the animals. The clinical uses of ultrasonography in female involve assessment of pubertal status, seasonal status of ovaries, stage of cycle, prediction of ovulation, pregnancy diagnosis, fetal viability, fetal age and sex, ovulation failure, ovarian and uterine tumors, follicular/luteal cyst, pyometra, mucometra, hydrometra, embryonic loss, postpartum involution, ovarian response to hormonal treatment.

Echotexture of normal ovarian structures

(i) Ovaries: The ovarian stroma appears as a mixed echotexture of hypo- and hyper-echoic display. Various types of structures can be imaged within the stroma depending on the physiological status of the ovary. In a small, inactive ovary the cortex can be seen to contain small anechoic (black) follicles (2-8 mm in diameter) whereas, medulla appears free of follicular activity. In a large, active ovary the differentiation in two zones is less distinct, and stroma is imaged as narrow.

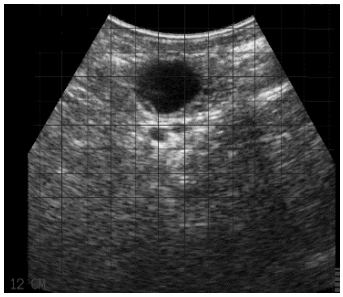
(ii) Follicles: Ultrasound is a more sensitive method than palpation per rectum for detecting and measuring ovarian follicles especially, those within the ovarian stroma. Future use of computer assisted image analysis may improve the diagnostic potential of

ultrasound to determine the health of a large follicle in a single examination. Ovulation is detected by ultrasonography as the acute disappearance of large follicle (9-20 mm) that was present at a previous examination. Several studies have been conducted to test the superovulatory response of various treatments. If an embryo transfer donor has failed to respond a standard superovulation regimen, the use of ultrasonography to characterize the activity of the dominant follicle prior to beginning FSH treatment may be beneficial.

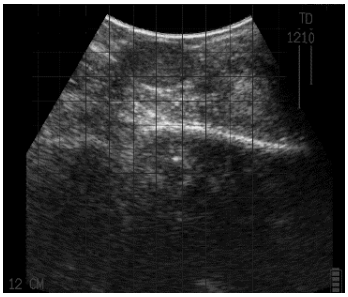
(iii) Corpus luteum: The ultrasonic detection and evaluation of corpus luteum (CL) provide valuable information to the diagnostician and biologist. The presence and stage of the luteal gland cannot be ascertained readily during the developing and regressing stages by trans-rectal palpation. Progesterone assays are not convenient for immediate consideration. Therefore, ultrasonography renders the immediate detection and evaluation of luteal gland. Ultrasonographic detection of CL may be more sensitive than detection by palpation but this is dependent on the experience of individual performing rectal palpation.

The CL in buffaloes is smaller than cattle in size, deeply embedded and has less pronounced ovulation papilla. Palpation of CL by per-rectum is thus difficult and ultrasonography provides correct picture of ovarian status. The echogenicity of CL depends on the stage of CL development. A mature active CL appears as large circular structure with a relatively homogeneous echotexture. The young, newly formed CL (corpora haemorrhagicum) is difficult to distinguish in its first four days of life, being imaged as a hyperechoic folded structure with a faint dark surrounding line. By six days post-ovulation, the CL is well defined in outline and this appearance will persist until 16 days post-ovulation. If pregnancy does not occur, the CL will regress and appeared as hyperechoic, heterogeneous structure with flattening of the outline. The presence of central cavity (lacuna) within the CL is a common feature. These cavities are distinguished from follicles by non-spherical, often lobulated appearance and by surrounding borders of luteal tissue. A CL with a fluid filled cavity is a normal condition and usually replaced by a dense, solid core of luteal tissue late in estrous cycle or during the first 25 days of pregnancy.Ultrasonography may provide a better method of evaluating CL in embryo transfer recipient. It is recommended that if there is a question

about suitability of CL after performing rectal palpation, the ovary can be scanned with ultrasound and decision made on whether to transfer to that recipient.



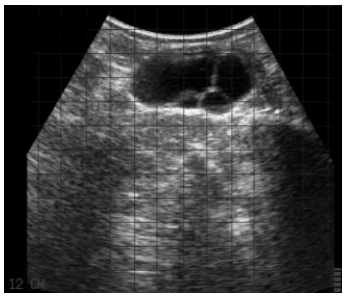
Dominant follicle



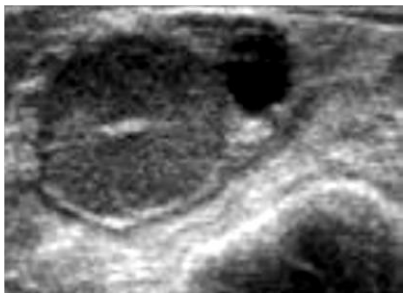
Mature CL

Echotexture of abnormal ovarian structures

In contrast to the natural and frequent occurrence of fluid filled cavities in the corpus luteum after ovulation, pathogenic cysts also form following failure of ovulation. Cysts are common in post-partum cows and buffaloes. Since these structures are anovulatory and may be persistent (≥ 20 mm). They are considered pathologic and are a source of transient infertility. Some cysts form a distinct luteal lining and are called luteal cyst, whereas others form little or no obvious lining and are called follicular cysts. Variation in the amount of luteinization of the cyst wall is difficult to assess by rectal palpation. The treatment of cows with ovarian cyst is dependent upon an accurate diagnosis of the condition and in particular whether the cysts are follicular or luteal .The failure to detect luteinization of follicular cyst by palpation per-rectum leads to unnecessary treatment in many cows. The therapeutic success can be confirmed quickly by visualization of cyst with ultrasonography. Thus, early diagnosis of cysts by ultrasonography helps in guiding for appropriate treatment and for preventing economic loss. Ultrasonography provides a method for measuring wall thickness and is valuable for diagnostic purposes. A firm thick walled (>3 mm) structure is diagnosed as luteal cyst and a soft, thin walled (< 3mm) structure as a follicular cyst.



Follicular Cyst



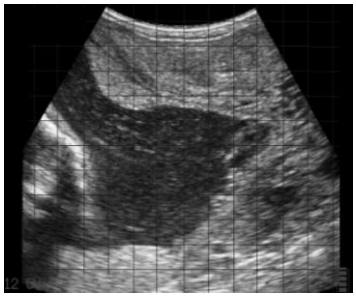
Luteal Cyst

Echotexture of female tubular genitalia

The cranial portion of the vagina is normally observed as a hyperechoic line close to the transducer face, but when it is fluid filled, it is seen to have an ovoid, anechoic lumen with enclosing hyperechoic lines. Various changes in vagina that occurs during estrous cycle can be visualized by ultrasonography. Vaginal fluid first increases on day 17 (equivalent to 4 days before ovulation) and decreases to base line (by day 6 or 7) after ovulation. The imperforate hymen (persistent hymen) can also be visualized through ultrasonography as accumulation of fluid in cranial vagina. The cervical rings appear as hyperechoic and fluid (anechoic) between them are more distinct. The cervix is thicker during estrus than during diestrus. The zigzag course of the cervical canal can be differentiated by rotating the transducer, with the identification of external os and portio-vaginalis within the cranial portion of the vagina.

Ultrasonic appearance of the uterus of the cattle and buffaloes is dependent on stage of the oestrous cycle. Variation in the appearance of the uterus involves changes in endometrial thickness, vascularity and the presence of intraluminal fluid. The Ultrasonographic appearance of abnormal uterine fluid can vary from anechoic fluid with floating particles (referred to as 'snowy specks') to homogenous, purulent exudates that can appear similar to the echogenicity of the surrounding uterus. In endometritic uterus, the fluid containing echogenic particles can easily be distinguished from the clear anechoic fluid of the peri-ovulatory period or early pregnancy. The presence of a thickened uterine wall associated with endometrial infection can also be identified with ultrasound. In the animals diagnosed with pyometra the fluid contain diffuse, echogenic particles within the distended uterus and a thickened uterine wall. The viscous fluid may resemble the uterine tissue but can be distinguished by the flowing motion of the

exudates within the lumen. Mucometra and hydrometra are often associated with segmental aplasia of the uterus and thin walled uterus appears to be full of echogenic particles. Ultrasound offers an objective method to assess treatment progress and to differentiate tissue characters associated with pathology of the reproductive tract.



Pyometra in buffalo

Pregnancy diagnosis

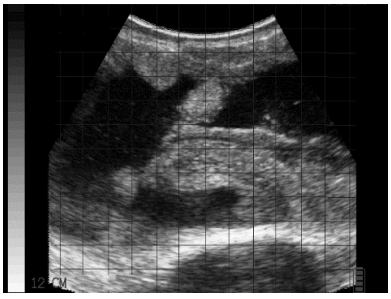
Under most on-farm conditions pregnancy diagnosis can be rapidly and accurately diagnosed using ultrasound as early as 26 days post AI. Pregnancy confirmation at early stage allows pregnant animals to be moved to a separate management group and managed less intensively (continued heat checking and/or a recheck 60-90 days later is highly recommended as there will be a normal 60 % pregnancy wastage or loss between 21 and 60 days post conception). It also allows open cows and buffaloes to be short cycled and re-inseminated or set up as recipients, decreasing the number of days open. Thee embryo proper is defined as a distinct echogenic structure within the non-echogenic, fluid filled vesicle. Presence and viability of the embryo initially can be confirmed by the detection of a heartbeat as early as 19 to 24 days of gestation. The embryo initially appears as a short, straight echoic line (20-22 days), later becomes C-shaped (22-30 days) and finally, by 30-32 days of gestation assumes an L shape. The potential advantages of using ultrasonography for pregnancy diagnosing are that the presence of an embryo can be detected earlier than by palpation per rectum and that direct physical manipulation of the gravid reproductive tract is not necessary with ultrasonography. The latter fact should reduce the risk of inducing embryonic mortality. Use of ultrasonography rather than per rectal palpation may also improve consistency of early (<45 days) pregnancy diagnosis by reducing the variation in accuracy among practitioners. The efficiency of detecting

early pregnancy with ultrasound is markedly increased when the embryo can be detected more easily. Although the embryo can first be detected between the days of 19 and 24 of gestation, when scanning large number of cattle, it is most practical to scan females which are expected to have embryos >24 days of age.

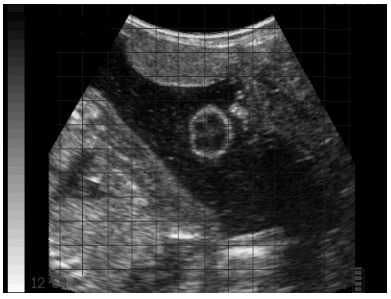
Determination of fetal viability and age

The growth of embryo proper from day 20-60 can be characterized and determined through ultrasonography when the characteristics such as the heart beat (day 22), spinal cord (day 28), placentomes (day 35), split hooves (day 44) and ribs (day 52) first become detectable. Measurements of crown rump length, head diameter and trunk diameter are the easiest predictive measurements to use for estimation of gestational age. In addition, the use of these measurements in formulas to estimate age results in the least variation between the estimated and actual ages. Crown rump length is that measured from the tail head to the greater curvature of the skull. Head and trunk measurements are recorded at their maximal diameters. A cross sectional or frontal presentation is required to record head and trunk measurements.

Macerated fetuses may appear as distorted images surrounded by purulent fluid characterized by anechoic background fluid containing echogenic particles. Degenerating embryonic tissues within the vesicle increases the echogenicity of the amniotic fluid surrounding the embryo, which also may appear distorted. Mummified fetuses often appear only as a poorly defined echogenic intrauterine mass without surrounding fluid. Occasionally, the bones may be identified as dense echogenic tissues shadowing the tissue below. A thickened uterine wall may also be apparent.



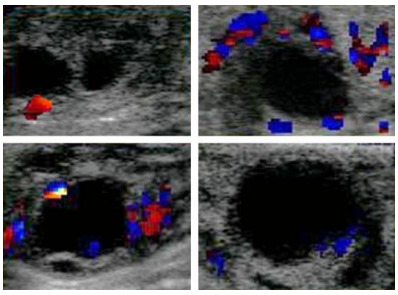
Abnormal pregnancy (Fetal maceration at 3 Months)



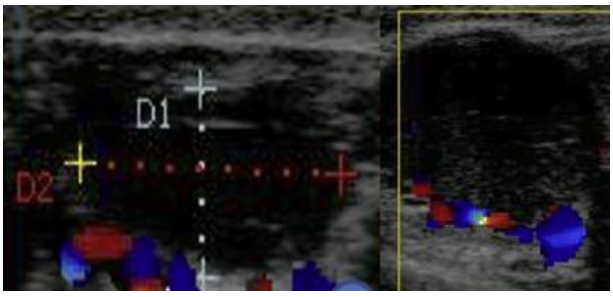
Fetal loss at 45 days of gestation in buffalo

Color Doppler ultrasonography

Color Doppler ultrasonography is a tool for evaluating vascularity of an organ or structure. For ovarian examinations, it allows visual observation of the blood flow in a demarcated area in the wall of preovulatory follicles, within the corpus luteum and changes in the uterine blood circulation in cows during the estrous cycle. The local blood flow using color doppler ultrasonography in individual ovarian follicles and the corpus luteum (CL) in the cow is closely related to follicular growth, atresia and ovulation, CL growth, maturity and its regression. Normal pregnancy is much associated with high vascularization of preovulatory follicle.



Color Doppler images of dominant follicle



Color Doppler image of mature CL

Suggested readings:

1. Ginther O J. 1998. Ultrasonic imaging and Animal Reproduction: Cattle. Equiservices, Cross Plains, Wisconsin, U. S. A. pp. 29-70.
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Recent Therapeutic Strategies for Anestrus in Dairy Animals

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Reproduction plays a vital factor in determining the efficiency of animal production. But there are certain problems affecting the livestock production which further directly affects farmer's income. Anestrus is one of the most commonly occurring reproductive problems in cattle and buffalo in India. 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. It is observed in post pubertal heifers, during pregnancy, lactation and in early postpartum period in adult animals. In heifers, it poses a herd problem possibly due to low plane of nutrition, stress of seasonal transition or extremes of climatic conditions. A large variation on incidence of anestrus has been reported in literatures depending upon species, breed, parity, and season, level of nutrition, managemental conditions, and geographic environment. Its incidence in heifers has been reported between 12.37 to 64.66 per cent. Incidence of anestrus is higher in adult cattle and buffalo than the heifers.

Anestrus leads to economic losses through increased inter-calving interval, poor net calf crops, production loss, treatment expenses and cost of replacing mature animal with first calving heifer. Long anestrus periods due to cessation of cyclic activity, due to smooth/quiescent ovaries or without palpable follicle and CL is known as true anestrus. False anestrus may be physiological (lactational, gestational and open days) or pathological (due to pyometra and chronic metritis). Anestrus is the most common single cause of infertility in buffaloes. Expression of overt signs of estrus is greatly affected by heat stress in buffaloes. In comparison to cows, buffaloes have lesser number of preantral and antral follicles, smaller sized pre–ovulatory follicle and greater tendency of follicular atresia which might be responsible for high incidence of anestrus in buffaloes. Broadly, anestrus is multi causative factors associated problems with the failure of animal to exhibit estrus. It may be prepubertal or postpartum. It may be due to:

- **Congenital abnormalities** like ovarian agenesis/hypoplasia, free martin and inter-sexuality.
- **Retention of Corpus luteum (CL)** due to uterine pathological conditions like Pyometra, chronic metritis and fetal mummification, fetal resorption, maceration etc.
- **Physiological anestrus:** Anestrus may be normal physiological after 45 days post parturition because this period is required for normal involution of genitalia. The high levels of placental and ovarian steroids i.e. estrogens and progesterone prevailing during late gestation exert a negative feedback effect on the hypothalamic–pituitary-gonadal axis.
- **Gestational anestrus:** If the animal is pregnant, then animal will not show estrus. But sometimes in 5-10% of cases the pregnant animal also exhibits estrus which is termed as gestational heat. It is normally observed in first trimester of gestation.
- **Nutrition:** Nutritional status of animals affects the follicular growth, maturation and ovulation. Under nutrition is the one of the most prevalent cause of anestrus in heifers. Extended postpartum period of anestrus (>150 days) are usually observed in cattle probably due to shortage of feed and good quality fodder. Reduced feed intake during late gestation or/and early postpartum period or negative energy balance (NEB) due to very high metabolic load following parturition especially in high yielders delays postpartum restoration of LH pulsatility, resulting into prolonged postpartum anestrus. In addition to NEB, the deficiency of minerals like calcium (Ca), phosphorus (P), copper (Cu), zinc (Zn) and manganese (Mn) are also associated with anestrus. It is well established that minerals play an intermediate role in the action of hormones and enzymes at cellular level and its deficiency ultimately affect the reproductive performance of female. Due to poor nutrition in heifers there may be delayed puberty or ovarian atrophy. Genitalia are poorly developed or infantile. In lactating animals it leads to small and smooth ovaries. Modern feeding and managemental practices also accentuate the problem in commercial dairy farms. Incidence of anestrus though varies in the different managemental system but it is more in buffalo than the cattle, and especially during summer.
- **Hormonal Imbalance:** If from the hypothalamus, pituitary and ovaries proper amount of hormones are not produced may lead to anestrus. On rectal examination the

genitalia is properly developed but ovaries of such animals are smooth. No CL is present.

- **Silent heat:** In some cases, the signs are exhibited but not observed or poor expression of heat symptoms may be there. The condition is termed as Silent heat. It is very common in buffaloes particularly during summer. The animal is normal cyclic but external signs (bellowing, frequent micturition) are not prominent because low level of estrogen hormone which is responsible for external signs. There will be history of slight vaginal discharge. On rectal examination either follicle or CL is observed on the ovary.
- **Heat Stress:** Due to heat stress the external signs of heat are poorly expressed and it is common in buffaloes during summer. During summer there is more secretion of ACTH and less secretion of LH.

Diagnosis of anestrus

- *History:* Based on the information viz., failure of displaying the overt signs of estrus by the animals after attaining puberty or 60–90 days post-partum; symptoms of estrus shown with cyclicity which subsequently ceased and revert in to anestrus. Such cases are diagnosed when presented for pregnancy diagnosis. Many times, livestock owner's complaint that they are not able to detect estrus or have not seen any signs of estrus in that particular animal since long.
- *Progesterone estimation:* True anestrus is usually characterized by a lack of ovarian progesterone production. Presence of basal level (0.5–1 ng/ml) of progesterone in the blood samples at an interval of 8–10 days further confirms the diagnosis. If the concentration of progesterone is more than 1ng/ml, it is suggestive of presence of corpus luteum and anestrus in such situation might be due to unobserved estrus/silent estrus/persistent corpus luteum.
- *Per Rectal examination:* Pregnancy can be a prominent cause of anestrus and therefore must be ruled out by careful examination of ovary and uterus when any animals present for gynecological examinations. On per rectal examination, ovaries are smooth, small and inactive with the absence of corpus luteum in true anestrus cattle and buffaloes however, follicles may develop up to prematuration stage and get atretic

Ghuman et al., 2010). Functional corpus luteum can be palpated in case of silent estrus/unobserved as well as in anestrus due to persistent corpus luteum.

- *Ultrasonography:* Ovarian pathology which is not accurately determined by per rectal palpation can be visualized by ultrasonography. Different stages of follicular growth and type of anestrus can easily be detected by ultrasonography.

Treatment of anestrus

- Properly examine the animal and if animal is suffering from congenital abnormalities, then cull the animal.
- If there is uterine pathology, treat it accordingly.
- *Lugol's iodine paint*: Painting of Lugol's iodine on posterior part of the cervix causes local irritation and brings about reflux stimulation at anterior pituitary for secretion of gonadotrophins and consequently cyclicity. It is an irritating solution (0.5 to 1.0%) causes hyperemia of uterine mucosa resulting into degree of iodine absorption from uterus. The absorbed iodine probably increases the metabolic rate of body through stimulating the thyroid hormone secretion. Increased metabolic rate trigger the ovarian functions by enhancing the energy utilization.
- *Non hormonal treatments; plant based heat inducers*: Plants synthesize varieties of phyto-chemicals 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. Many plants such as Murraya koenigii (curry leaves), Nigella sativa (kalonji), Abroma augusta (Ulatkambal), Saraca asoca (Ashoka), Trigonella foenum–graecum (Methi), Bambusa aruninacea, Carica papaya, Asparagus recemosus, Leptadenia reticulate, Courupita guianesis, Pergulacia daemia, Semecarpus anacardium cucumber, and jute plants either alone or in combinations have been fed to treat the anestrus animals with variable response on induction of estrus.
- *Improve the nutritional status of animals:* For this supplement the ration with 50-60g of mineral mixture daily for 2-3 months.

- *Hormonal therapy:* After this induce the estrus by the hormonal treatment. For this we can give:

A. Estrogen: In presence of dominant follicle, estrogen administration results in expression of estrus and ovulation because of its positive feedback effect over pituitary for LH surge. For this reason, it has been used to induce ovulation and to reduce postpartum anestrus period.

- One or two doses of i/m inj estradiol (3–10mg) or estrone (5–15mg) at three days interval can be used to regresses the retained CL associated with pyometra, mummification and mucometra.
- Give estrogen 10 mg i/m. Animal will come into heat within 2-3 days after injection. It should never be given in lactating animals but can be used in heifers.
- Side effect: Prolonged dose of estrogens leads to cystic ovaries, abnormal motility and peristalsis of oviduct results in infections to the ovarian bursa through oviduct, causing ovaritis and adhesions. In lactating animals, cause drastic drop in milk yield.

B. FSH: Follogon 1000 I.U.i/m. Animal will come into heat within 3 days. Disadvantages of FSH/PMSG/eCG include: twinning/triplets; lead to drop in milk yield.

C. Progestagens/Progesterone: Now a day's progesterone therapy is widely used for estrus induction. Exogenous administration of progesterone mimics the luteal phase of the estrus cycle by exerting negative feedback effect over hypothalamus and pituitary for LH release. Upon withdrawal of progesterone, the normal follicular phase of the cycle is stimulated. However, for such treatment seem to be effective, abrupt decrease in progesterone level is required at the end of treatment. The progesterone may be natural or synthetic and used in various protocols.

- *Injectable progesterone*: In this 50-100 mg of progesterone (Proluton depot/ Duraprogen) is administered for 12-14 days. After the last injection animal will exhibit estrus within 3-5 days.
- *Oral Progesterone (MGA)*: It is synthetic progesterone known as Melengesterol acetate. Dose is 0.5-1 mg in feed daily for 12-14 days. The estrus is exhibited within 5 days after last feeding.

- *Progesterone releasing intra-vaginal device (PRID)*: Device consist 1.55gm progesterone, insert into vagina for 7-12 days and inject PGF2α one day before or at the time of removal of device, animal will come into heat within 24-72 hours.
- *Controlled internal drug release device (CIDR)*: This is an intravaginal 'T' shaped device/insert is impregnated with 1.38 grams of progesterone in elastic silicone molded over a nylon spine. It is left into the vagina for 7-12 days and PGF2α is injected at the time of removal of device or one day before the removal of implant, animal will come into heat within 24-72 hours.
- *TRIU-B (Virbac India)*: Each device comprises of 3 medicated rings (green colour) containing Progesterone IP 186 mg each and one additional ring (pink colour) with Progesterone IP 400 mg.
- *Synchromate-B and Crestar ear implants*: Synchromate-B contains 6mg norgestomet in combination with an injection of 5mg estradiol valerate and 3mg of norgestomet. Crestar ear implant contains 3mg norgestomet in combination with an Injection of 5mg estradiol valerate and 3mg of norgestomet. The implant is removed after 9-10 days and give better estrus synchronization within 24-72 hours after removal.

D. Ovsynch protocol/G-P-G regime: On day 0 inject GnRH analogue (Buserelin acetate; Receptal) 20µg i/m. Inject $PGF_2\alpha$ (Dianoprost tromethamine; Lutalyse) 25 mg i/m and again on day 9 give GnRH analogue (Buserelin acetate; Receptal) 20µg i/m. The heat is exhibited within 24-48 hours after the last GnRH injection. Acyclic animals respond much better to Ovsynch protocol than anestrus animals.

E. Gonadotrophins Based Treatment: Pregnant mare serum gonadotrophin (PMSG) or equine chorionic gonadotrophin (eCG) is strong stimulator of ovarian activity because of its predominant FSH like activity. Therefore, it has been used extensively for superovulation. PMSG prevent and reverse the process of atresia in small follicle, hence its use for management of anestrus in buffaloes in low doses could be used satisfactory as follicular atresia is very common in buffaloes.Human chorionic gonadotrophin (hCG) has also been used for management of anestrus with fair degree of success.

F. Prostaglandin Based Treatment: Prostaglandin (PGF2α) is the treatment of choice for persistent corpus luteum and sub estrus. PGF2α is only effective between days 6–16 of the cycle and in the presence of active corpus luteum. An intramuscular injection of

25mg (total dose) of natural PGF2α or 250 to 500 micrograms of synthetic ones is required to regress the CL in both cattle and buffaloes. However, a lower dose of PGF2α (5mg) are also effective to regress the CL through intra–vulvo–submucosal (IVSM).

G. **Insulin Based Treatment:** The recommended dose is 0.25 IU/kg BW s/c for 3–5 days. Use of GnRH or eCG pretreated with insulin has shown promising results for management of anestrus cattle and buffaloes. Insulin enhances the follicular growth in true anestrus buffalo which is prerequisite of GnRH to be effective. Single i/m inj of PMSG (500 IU) was combined with subcutaneous injections of insulin @ 0.25IU/Kg body weight for five consecutive days.

H. **Anti–Prolactin Based Treatment:** Hyper–prolactinaemia has been reported during summer in buffaloes that could be one of the reasons for summer anestrus in buffaloes. With this assumption anti–prolactin drug such as bromocriptine has been tried.

Melatonin is also known to suppress prolactin secretion. Moreover, melatonin has been reported as stimulator of both GnRH and gonadotrophin secretion in buffaloes. As the plasma concentration of melatonin is low during summer, it has reported estrus induction and ovulation in all melatonin treated summer anestrus buffalo heifers using melatonin implants, however, time taken to induce estrus and ovulation was highly variable (4–36 days).

Treatment of silent estrus/unobserved estrus: Ask the owner about day of vaginal discharge and give $PGF_2\alpha$ after 10-12 days of vaginal discharge or palpate the CL and give $PGF_2\alpha$. Animal comes into heat with 72 hours after PG. Treatment may also include double shot of $PGF_2\alpha$ injections at 10 days apart in cases of improper history of heat in cyclic animals.

Prevention

- By maintaining the healthy status of the animals by adopting efficient farm managemental practices.
- Nutrition is probably most important factors, affecting ovarian activity. Special attention must be given to prevent the negative energy balance in high yielders. It can be achieved by providing adequate ration during pre– and postpartum period. The

supplementation of vitamins, minerals and antioxidants in feed appeared to be promising in restoration of cyclicity

- As suckling decreases the LH pulsatility and prolongs the postpartum anestrus period, weaning could be one of the effective managemental tools to reduce postpartum anestrus period following restoration of pulsatile secretion of LH in postpartum animals.
- Biostimulation (induction of cyclicity through introduction of males into a group of females) activates LH secretion followed by ovulation in females through olfactory and sensory cues.
- Certain managemental practices such as efficient detection of estrus, routine pregnancy diagnosis (40–60 days post breeding), prevention of occurrence of post-partum uterine infections and periparturient diseases (ketosis and mastitis), regular deworming and synchronization of estrus especially in buffaloes where heat detection is more difficult, could be advantageous in preventing the occurrence of anestrus.

Conclusion

Anestrus is a multi-causative factors associated problem affecting livestock enterprise to a great extent. Diagnosis of the condition needs to be prompt and at the earliest to prevent its occurrence for effective treatment. As such there is no single panacea to correct it.

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Management of Repeat Breeding in Cattle and Buffaloes

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Cattle and buffalo play an important role in maintaining a sustainable food production system in India. The productivity of bovine, however, remains low largely due to poor management of health, nutrition and breeding. The major problems faced by breeders and farmers include poor reproductive efficiency and prolonged inter-calving intervals. Clinical evaluations have shown that repeat breeding (RB) is the major cause of infertility in bovine. A repeat breeder is generally defined as any animal that has not conceived after three or more services associated with true estrus. The incidence of repeat breeding is high in cows compared to buffaloes (approximately 19 *vs.* 9%, respectively). Repeat breeding syndrome is responsible for long service period and inter-calving interval thereby causing low milk and calf production resulting in to greater economic losses to dairy industry.

The cause of repeat breeding is unclear and multifactorial. Hormonal insufficiency and dysfunction contribute about 40.1% causes of repeat breeding. Prolonged duration of estrus, extended follicular phase, delayed luteinizing hormone (LH) surge and thus delayed ovulation, late postovulatory rise in plasma progesterone considered to be most prominent factors responsible for repeat breeding. Failure of fertilization is mostly associated with poor heat detection by farmers, improper estimation of fixed-time artificial insemination. It is also due to the abnormalities related to poor semen quality. Either failure of fertilization or early embryonic death is considered to be major pathogenesis of repeat breeding animals. Other risk factors include tubal obstructions, early or late embryonic death, poor breeding and management techniques including genetic, nutritional and infectious factors. Therefore, broadly major causes of repeat breeding can be classified as:

a. *Genetics:* Chromosomal or genetic abnormalities adversely affect bovine fertility. Factors like inbreeding, aged gamete also provoke genetic defects leading to repeat breeding. Certain breeds like Holstein and Jersey show high incidence of repeat breeding.

b. *Genital defects:* Anatomical or functional alterations in oviducts, ovarian bursa, uterine horns and body, cervix either due to injudicious handling while performing artificial insemination (AI), pregnancy diagnosis (PD) or during handling of dystocia can cause gestational failure and repeat breeding syndrome.

c. *Age:* Higher incidence of repeat breeding has been seen in old animals. It is observed that fertility in dairy cows gets better after the 1st or 2nd parity, and then declines from the 4^{th} and 5^{th} lactation onwards.

d. *Body conditioning and nutritional causes:* The fertility of the dairy animals is associated with their body weight. As per the recommendations of ICAR, dairy cow heifers should achieve above 250 kg and buffalo heifers must attain above 275 kg before breeding for optimal reproductive management. Underweight animals show poor rates of conception. In India, nutritional deficiency is considered as most important factor causing reproductive failures in bovine. Majority of animals show infertility due to unbalanced feeding (energy, fat, protein, vitamins and minerals). The trace minerals deficiency prevails in Indian dairy herds that lead to problem of repeat breeding in dairy animals.

e. *Uterine infections:* The uterine environment encourages the normal embryonic development. Hence, any disorders or defects like uterine infections, endometritis, pyometra, metritis etc. adversely affect fertilization or survival of the embryo causing embryonic death which is also one of the major reasons for repeat breeding. Periparturient problems like dystocia, retention of fetal membranes, genital prolapse etc. lead to delayed uterine and cervical involution causing uterine infections, delayed ovarian rebound, high embryo mortality and repeat breeding.

f. *Ovarian dysfunctions:* Various ovarian dysfunctions like cystic ovarian degeneration (COD), luteal deficiency resulting into progesterone deficiency, incomplete luteolysis, delayed ovulation may also provoke repeat breeding syndrome. The problem of ovarian cysts (follicular and luteal) is quite common in exotic high yielder cows and is considered a major reason for reproduction failure.

g. *Male factors:* Any disorder at any action involving bull preparation, artificial vagina preparation, semen collection, semen processing, storage, thawing, post-thaw handling of semen may also result into repeat breeding syndrome.

h. *Managemental issues:* Any housing issue leading to environmental stress, lameness, inadequate estrus detection, incorrect timing of insemination in relation to stage of estrus, improper technique of AI etc. may cause repeat breeding among dairy animals.

Management of repeat breeding

Certain issues related to genetics and anatomical defects are unavoidable and untreatable. Therefore, such problems must be identified early in life and culling of affected animals should be considered following thorough examination to reduce the economic losses. For example, ovaro-bursal adhesions (OBA) can easily be diagnosed by rectal examination and if the case is bilateral then only solution is culling, whereas estrual animal with unilateral OBA can be inseminated if its unaffected ovary possesses a good dominant follicle (DF). Many experts recommend deposition of semen in the uterine horn epsilateral to normal ovary with good sized (10 mm or more) DF. If any animal has kinked cervix and passing of AI gun through cervix is difficult then natural mating can be recommended to inseminate that animal. It is said that such animal if carry the pregnancy to term and parturates normally then its cervix may get normalized afterwards.

For adequate reproductive management, dairy animals must be maintained in a good body condition. A BCS of < 2 and >4 severely affects the capability of animal to conceive. Thus, it is recommended to maintain a BCS between 2.5 to 3.5/5.0 basis for

optimal fertility. To maintain a good BCS, animals must be dewormed properly (at least 3 times/year) with broad spectrum dewormers otherwise deficiency of essential nutrients may occur. The ration must contain green fodder (approximately 40 kg/day/animal), dry fodder (2-5 kg/day/animal) and 2-3 kg of balanced concentrate feed if heifer or dry animal and according to milk yield if lactating. In general, if green fodder is available *ad lib* then 1 kg concentrate feed is sufficient as a maintenance ration for a buffalo producing 5 kg and a cow producing 7 kg of milk per day. Afterwards, add 1 kg concentrates for every 2 kg and 2.5 kg of extra milk produced by buffalo and cow, respectively. Mineral mixture supplementation should be an integral part of diet @ 2% of ration or 30-50 g on daily basis. Provide the animals clean and fresh water to drink round the clock.

To control the uterine infections, periparturient care of the pregnant animals is most important. Provide anti-oxidants (vitamin E, Se), vitamin A, D, H etc. during transition period. Keep a vigil at calving. Give high energy and calcium enriched diet immediately post calving to reduce incidence of retention of fetal membranes, milk fever, ketosis etc. It is believed that weekly administration of vitamin E and prostaglandin injections post calving accelerate the process of uterine involution. If uterine infection is diagnosed then treat the animal with broad spectrum antibiotics such as cephalosporins (Ceftiofur), tetracycline, metronidazole, enrofloxacin or on the basis of CST results. Always prefer systemic administration of drugs over local route. In general, to miss an estrous cycle after treatment is a preferred way to clear any remaining subclinical infection by endogenous estrogens. Injections of prostaglandins are preferred in cases of pyometra or persistent corpus luteum (CL) to clear the infections. In some cases, single intrauterine administration of 0.25 – 0.5 % lugol's solution or 100 mcg LPS is effective.

To reduce the environmental stress, avoid overcrowding in the sheds and provide both concrete as well as dirt floor. Adopt a practice of loose housing system. Provide as much as cool climate to the animals during summer especially to crossbred animals. Heavy plantation around the farm, sprinkling water, ventilation, bathing and roof painting will help keep the animals cool and healthy in summer. Always keep a check on ectoparasites. Manage lameness, mastitis and other diseases.

Other managemental issues like improper maintenance of records, poor detection of estrus, improper timing and technique of insemination, and poor semen quality are also responsible for high incidence of repeat breeding among dairy animals.

Poor estrus detection is one of the most important factors responsible for reproductive losses in a herd. Different methods to improve estrus detection include: proper identification of animals (Good branding, large ear tags etc.), regular observation of animals (observe the animal for 15-30 min and 3-4 observations @ 6 h interval), adequate provision of light especially at night, heat expectancy charts **(s**pecial calendars used to record information), pressure-sensitive mount detectors, tail chalk, crayon, or paint, chin ball markers, heat watch, pedometer, cow scout, vaginal electrical resistance, ultrasound scanning, progesterone assay recording of vaginal temperature, trained dogs and rectal palpation. But the most important way is visual detection, thus experienced person should do that job.

Insemination should always be preferred at least 10-12 h before ovulation. Optimum time to inseminate is between 6-18 h after onset of estrus (standing estrus). Usually farmers report the time to the technician, when they observe the heat signs. The technician inseminates the animal if it is presented to him in hospital, else he goes to inseminate the animal at farmer's doorstep whenever he gets time. Wrong timing of insemination may lead to fertilization or conception failures either due to aged ovum or spermatozoa. We should inseminate the indigenous cows and buffaloes according to A.M.-P.M. rule i.e. if animal comes in heat in morning, it should be inseminated in the same day evening and if it shows heat in the evening then should be inseminated on next day morning. It is better to give double insemination in crossbreds that should be done with the gap of 12-24 hours after first AI.

Last but not least, selection and timing of hormonal interventions to treat various conditions viz. luteal deficiency, COD, anovulation, delayed ovulation, persistent CL in absence of uterine infections are quite important to combat repeat breeding syndrome in dairy animals. For example, use of a luteolytic agent such as PGF2α, or an analogue, which causes the regression of the CL is successful when animals are bred to a detected

estrus. This method does not control the time of AI, as estrus detection continues to be necessary. If fixed time artificial insemination (FTAI) is performed after PGF2α, a low pregnancy rate is expected. Prostaglandins are only effective if administered between days 8 to 17 of the estrous cycle. In some cases of delayed ovulation an injection of GnRH (10-20 mcg) is given at the time of or 12 h before AI to cause timely ovulation.

Various GnRH based synchronization methods are also used to treat hormonal imbalance and to eliminate need of estrus detection in dairy animals. Most common GnRH based method is "Ovsynch" in which an injection of GnRH (10-20 mcg, intramuscularly in morning) is administered at a random stage of estrous cycle followed by an injection of PGF2α (Lutalyse 25mg or Cloprostenol 500 mcg, intramuscularly in morning) 7 days later. Ovulation is synchronized by a second injection of GnRH (10-20 mcg, intramuscularly in evening) given 2 days after PGF2α. The animals are then inseminated at a fixed time of 16 h post second GnRH injection. The first injection of GnRH induces ovulation of dominant follicle (DF) and causes emergence of a new follicular wave. The PGF2α injection induces regression of the spontaneous and/or GnRH induced CL and the second GnRH injection synchronizes the time of ovulation of the DF of the follicular wave that began growing after the first GnRH injection. However, there are certain stages of the estrous cycle when initiation of the Ovsynch program causes reduced pregnancy rates. Initiation of Ovsynch program during late stage of estrous cycle eg. after day 13 is a time during which spontaneous regression of the CL occurs prior to the time that PGF2α is administered at 7 d after the administration of GnRH. Such animals ovulate prematurely relative to insemination. Similarly, initiation of Ovsynch program during early stage of the estrous cycle eg. day 2-4 leads to low pregnancy rates. At this stage, spontaneous ovulation has already occurred, and the potentially new DF is too small to ovulate in response to GnRH administration. As a consequence, the DF at the second administration of GnRH is considered aged. Therefore, it is said that IF STAGE OF estrous cycle is known then 'Ovsynch' should be initiated during 5-9 days of estrous cycle.

The progestogens (CIDR) maintains high levels of progesterone in the female's system, even after regression of the corpus luteum and are quite successful in cases of

COD or inactive ovaries. Addition of progestins in the GnRH based protocols in the form of MGA (0.5 mg/d) feeding for 5 or 6 days after first injection of GnRH or intra-vaginal insert (CIDR) on the day of first GnRH injection till day of PGF2α injection during the protocol may yield satisfactory results. The primary benefit of inclusion of the CIDR in GnRH-based programs is that it guarantees that females will be exposed to progesterone during the period between day 1 and day 8.

Now days to further improve the conception rates, various modified GnRH based protocols viz. Doublesynch, Estradoublesynch, Presynch-Ovsynch, Presynch-Heatsynch etc. are preferred over traditional Ovsynch method.

In conclusion, repeat breeding is a potential cause of low fertility in bovine and high economic losses to dairy farmers. The etiology of repeat breeding appears to be multi-factorial. Proper identification of the cause of repeat breeding and its timely management is need of the hour to uplift the dairy farming in India.

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Recent advances in diagnosis and therapeutics of uterine infections in dairy animals

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The multifactorial uterine infections can affect almost half of dairy cows in a herd due to compromised immunity during the postpartum period. The diagnosis and treatment of uterine infections is important in dairy animals as their consequences are subfertility, infertility, increased veterinary cost, impaired production and reproduction traits leading to large economic losses to farmers. An infected uterus is a source of bacterial compounds and cytokines that spill into the systemic circulation, spreading inflammation to other organs. The conception rates are about 20% lower for cows with uterine infections that result prolonged calving to conception interval and there are 3% more animal culled because of failure to conceive. It has been reported that within 10 days after calving, around 21% of dairy cows develop uterine disease, such as metritis, due to the high numbers of pathogenic bacteria that ascend into the uterus postpartum. Consequently, around 40% of the cows are not able to clear bacterial pathogens efficiently and develop endometritis.

Metritis complex

Metritis complex is a term collectively used for the various post-partum uterine infections which includes; retention of fetal membranes (RFM), metritis, clinical endometritis, subclinical endometritis and pyometra.

Retention of fetal membranes (RFM)

RFM is defined as the failure of an animal to expel the fetal membranes within 24 hours of the end of parturition. Retained placenta is an alternative name used for RFM. There is some variation in the literature about the duration of retention that defines the clinical disorder. Some prefer to define retention as being for 12 hours; however, the timing is arbitrary, and most normal cows expel the fetal membranes within a few hours

of parturition. The incidence of RFM varies among herds, but is typically 5% to 10% of animals. The importance of RFM is that they are associated with reduced milk yield and an increased risk of metritis. It is a common complication of bovine parturition. The predisposition to infections of the uterus means that RFM are an important contributor to bovine infertility. Occurrence of RFM is associated with the failure of the normal processes of placental dehiscence and expulsion.

Metritis

Metritis is most common within 10 days of parturition. Metritis is characterised by an enlarged uterus and a watery red-brown fluid to viscous off-white purulent, uterine discharge, which often has a fetid odour. The incidence of metritis varies between breed, country, and herd. However, in some studies the incidence is as high as 40% of the herd. The associated clinical signs are used to classify the severity of disease, which varies from unapparent disease to fatal toxaemic metritis. Uterine infections can be evaluated by the physical characteristics and odor of the vaginal cervical mucus and a 0 to 3 ranking system has been proposed to evaluate the grade of metritis.

- Grade 1 metritis: It is characterized by having an enlarge uterus and an infection causing the cow to have a purulent uterine discharge within 21 days after parturition. Cows have no systemic signs of illness.
- Grade 2 metritis: It includes signs of illness like fever (>39.5 ◦C), decreased milk yield, and a fetid red-brown uterine discharge.
- Grade 3 metritis: It is also known as puerperal or toxic metritis, in addition to the aforementioned signs for Grade 1 and 2 metritis, is associated with decreased feed and water intake, and can be associated with development of the downer cow syndrome. Puerperal metritis is highly associated with dystocia, retained placenta, twin births, and abortion, which occurs during the first week after parturition.

Clinical endometritis

Clinical endometritis is defined as the presence of a purulent uterine discharge detectable in the vagina of cattle 21 days or more postpartum or a mucopurulent discharge detectable in the vagina after 26 days postpartum. The incidence of clinical endometritis is around 10% to 20%, with variation between breed, country, and herd. A simple grading system based on the character of the vaginal mucus is readily used to evaluate cows with clinical endometritis and is prognostic for the likely outcome of treatment.

Subclinical endometritis

Subclinical endometritis is characterised by inflammation of the endometrium in the absence of clinical signs of endometritis, which results in a significant reduction in reproductive performance. The inflammation is presumably associated with recovery of the tissues after metritis and clinical endometritis, trauma, or other non-microbial disease. Definition of subclinical disease is currently dependent on cytological analysis of samples that are collected from the surface of the endometrium by flushing the uterine lumen or by the use of an endometrial cytobrush. The proportion of animals affected varies widely among studies, ranging from about 11% to more than 40% of animals. Diagnosis relies on the proportion of endometrial cells that contain more than a defined proportion of neutrophils at specific times after parturition.

Pyometra

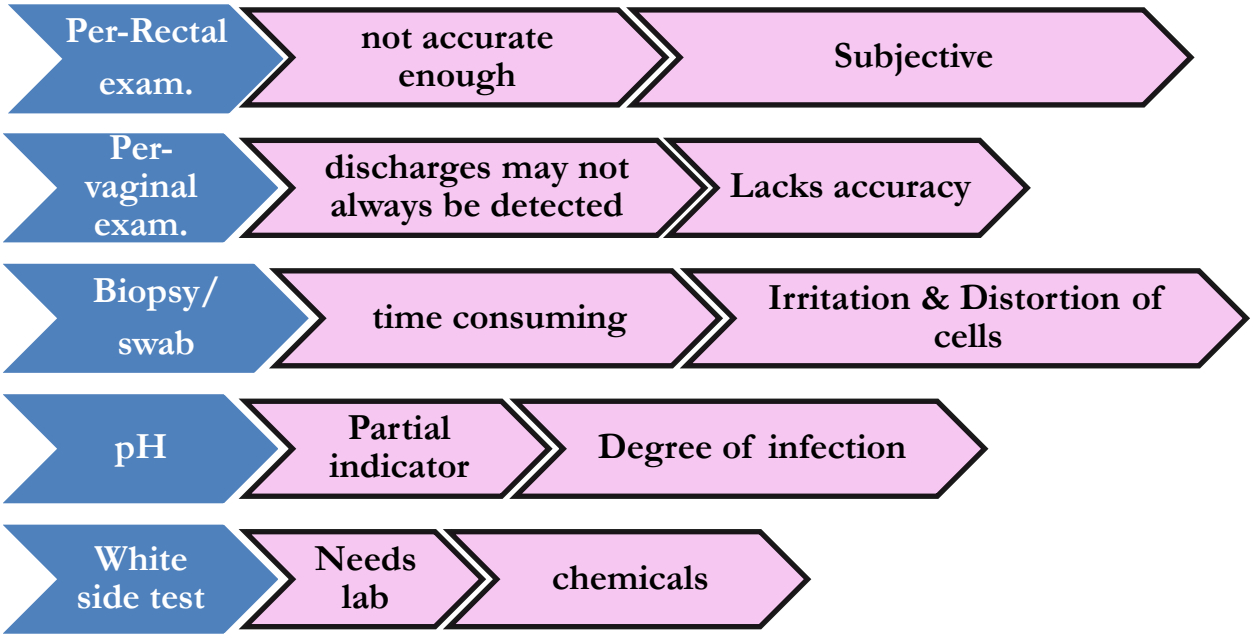
Pyometra is characterised by the accumulation of purulent or mucopurulent material within the uterine lumen, causing distension of the uterus, in the presence of a closed cervix and a functional corpus luteum (CL). Postpartum pyometra is uncommon and is thought to be caused by the growth of bacteria within the uterine lumen after the formation of the first CL.

Predisposing factors for uterine infections

The environment is an important determinant of susceptibility to several uterine diseases, and many risk factors have been associated with uterine disease. Indeed, environmental factors may be more important than genetic factors. The environmental risk factors most likely to cause uterine disease are associated with tissue damage. Trauma may delay uterine involution, keep the cervix open, and allow bacteria to access the underlying stroma below the protective epithelium. Obvious causes of trauma include: dystocia, a large male calf, stillbirths, twins, first parity, and induction of parturition. However, RFM are the most important risk factor for uterine disease. The necrotic material associated with RFM provides a favourable environment for bacterial growth in the uterine lumen, and the retained membranes obstruct the physical barrier provided by the cervix, and delay uterine involution. It is intuitive that the hygiene of the calving environment and the postpartum housing should be important for development of uterine disease. Uterine disease is associated with changes in metabolism after parturition or diseases that disrupt metabolism such as left displaced abomasum. Dairy cows are often under metabolic stress because they cannot consume enough food to meet the substantial extra demand for nutrients that are required for lactation. Larger herds tend to have more uterine disease, and disease is often more common in production systems where animals are kept in close associations.

Diagnostic tools

Conventional techniques



Advancements in diagnosis of uterine infections

- **Ultrasonography:** Ultrasonography has numerous advantages over other imaging modalities. It is non-invasive, free from radiation hazards, provides instant diagnosis, and determines shape, size, location and internal consistency of a structure. Ultrasonic appearance of the uterus of the cattle and buffaloes is dependent on stage of the oestrous cycle. Variation in the appearance of the uterus involves changes in endometrial thickness, vascularity and the presence of intraluminal fluid. The Ultrasonographic appearance of abnormal uterine fluid can vary from anechoic fluid with floating particles (referred to as 'snowy specks') to homogenous, purulent exudates that can appear similar to the echogenicity of the surrounding uterus. In endometritic uterus, the fluid containing echogenic particles can easily be distinguished from the clear anechoic fluid of the peri-ovulatory period or early pregnancy. The presence of a thickened uterine wall associated with endometrial infection can also be identified with ultrasound. In the animals diagnosed with pyometra the fluid contain diffuse, echogenic particles within the distended uterus and a thickened uterine wall. The viscous fluid may

resemble the uterine tissue but can be distinguished by the flowing motion of the exudates within the lumen.

- **Cytobrush technique:** with the help of cytobrush fitted in cytobrush assembly uterine sample is taken by rolling the cytobrush clockwise and anticlockwise and smear is prepared, fixed with methanol and stained with Giemsa stain. If PMN cell count is more than 10% then animal is said to be positive for sub-clinical endometritis. It is superior in all respects as more consistent and reliable method than the lavage method. It is useful tool and most reliable to evaluate sub-clinical endometritis in cows and more helpful to accurately diagnose endometritis in cows.
- **Cytotape:** Less distorted–fragmented cells and a significantly lower contamination with RBCs were reported using cytotape than cytobrush.
- **Metricheck device:** With the Metricheck device, significantly more cows were diagnosed as affected with endometritis than by examination with a speculum or a gloved hand. However, the discharges may arise due to vaginitis/cervicitis which leads to wrong interpretation of uterine health.

Therapeutic measures

Intrauterine vs systemic therapy

It has been observed that choice of whether intrauterine or systemic therapy is still a question before the veterinarians to treat the uterine infections. The systemic antimicrobial therapy must be used to treat the cases of RFM, metritis and pyometra while intrauterine treatment is preferred treatment of choice for endometritis as it is local inflammation and does not result in systemic illness.

Principles of antimicrobial therapy

Some clear principles underlie the choice of antimicrobial and/or antiseptic agents:

- It must be effective against the wide range of aerobic and anaerobic, Gram-positive and Gram-negative bacteria that are present.
- It must be effective within the microaerophilic environment of the uterus.
- Whether an effective bactericidal or bacteriostatic concentration can be achieved at the site of infection by the intrauterine route of administration.
- When the intrauterine route is used, the substance must be evenly and rapidly distributed throughout the uterine lumen with good penetration into the deeper layers of the endometrium.
- It must not inhibit natural uterine defence mechanisms, particularly the cellular component.
- It must not traumatise the endometrium. Several of the vehicles used in the formulation of pharmaceutical preparations can damage the endometrium. Examples include propylene glycol, which can cause a necrotising endometritis; oils, which can cause granulomata; and chalky bases, which can cause irritation and blockage of endometrial glands.
- Treatment must not reduce fertility by producing irreversible changes in the reproductive system.
- Treatment must be cost effective by enhancing fertility.
- Details of its absorption from the uterus and excretion in the milk must be known, so that appropriate withdrawal times can be followed. In consequence, several antibiotics are inappropriate.
- Nitrofurazone is an irritant and has an adverse effect on fertility.
- Aminoglycosides are not effective in the predominantly anaerobic environment of the infected uterus. Field trials have also provided evidence for a lack of effectiveness of these drugs in the treatment of endometritis.
- Sulphonamides are ineffective because of the presence of paraaminobenzoic acid metabolites in the lumen of the infected uterus.
- Penicillins are susceptible to degradation by the large numbers of penicillinase producing bacteria that are present in the infected uterus.
- Intra-uterine administration of tetracyclines may worsen subsequent reproductive performance due to irritation and pH damage to the endometrium.

Antibiotherpay

A wide range of antimicrobial agents has been used in the treatment of endometritis. Although parenteral antibiotic treatment is needed for metritis, it is generally considered to be preferable to treat endometritis via the intrauterine route. Provided an adequate dose of antibiotic is used, this will result in effective minimum inhibitory concentration (MIC) reaching the endometrium and being established in the intraluminal secretions. The latter point is important for the effective treatment of the disease because sub-therapeutic dose rates are frequently used. In organic herds, antibiotics are not often permitted, and so mild antiseptics may be used, such as 2% povidone iodine or a hibitane solution. Ceftiofur @ 1.1-2.2 mg per Kg body weight is drug of choice for parenteral administration while, cephapirin is drug of choice for intrauterine therapy. Flunixine meglumine @ 2.2 mg mg per Kg body weight is anti-inflammatory drug of choice to treat uterine infections.

Immunomodulators

- ***E. coli* Lipopolysaccharide (*E. coli* Endotoxin)**: 100-200µg of *E. coli* intra-uterine infusion increases the leucocyte count 100 folds. These are recommended for post AI and therapeutic purpose in multiple doses in the treatment of sub-clinical endometritis in bovines. In dystocia cases, instillation of 300 µg after relieving dystocia or caesarean prevents development of pyometra as well as subsequent endometritis.
- **Oyster glycogen**: 1-10 % oyster glycogen leads to lecocytosis in uterine lumen by 2-30 folds. Intra-uterine infusion of oyster glycogen 500 mg in 50 ml of vehicle leads to marked improvement in the non-specific uterine defense and endometrial histopathological pictures in acute and chronic endometritis.
- **Serum, Plasma and hyper-immune serum**: Addition of a small amount serum into uterus increases phagocytic ability in uterus. This is by presence of complement, antibodies and opsonin in the serum. 50-100 ml of autologous plasma for 3 days can be given in the uterus.

- **Granulocyte-Macrophage Colony Stimulating factor (GM-CSF)**: It is a lymphokine which helps in the differentiation and proliferation of certain hemopoitic cells and particularly help in formation of mature PMN colonies or macrophage or both. GM-CSF increases lyphocytic ability, inhibits their migration and makes them more adherent to the bacteria.
- **Inmodulen (LPS+ Propionibacterium granulosum)**: Recommended both intra-muscular and intra-uterine infusion.
- **Lysozyme**: Intra-uterine infusion of 2 mg of lysozyme in 50 ml PBS buffer or 5 % glucose solution increases INF alpha factor and cause acute phagocytosis. Besides, it also has antiviral efficacy and modulate TNF alpha synthesis without any mutagenic or allergic reactions. This can be used for prophylaxis as well as therapeutic purposes. Repeated infusions are given after 24 to 72 hours if necessary. Available commercial preparation: Lydium KLP TM (Nika Health Product Ltd.).

Hormones

- **Prostaglandin F2α:** If a functional CL is present then PGF2α is most successful treatment to be used. Pyometra is treated in cattle using PGF2α and after opening of the cervix it could be treated as like that of metritis.

Proteolytic enzymes

A combination of three proteolytic enzymes viz. chymotrypsin, trypsin and papain which act as biological scalpels and had fibrinolytic and proteolytic activity in the inflamed tissue results in breakdown of products of infection, damaged cells and tissues. The gram-positive and gram-negative bacteria, yeasts, and toxins contain proteins and lipids that are degraded directly by these enzymes leading to stasis in growth or death of bacterium. The pregnancy rate in subclinical endometritic cattle treated with proteolytic enzymes improved than non-treated ones.

Suggestive readings

- Veterinary Reproduction and Obstetrics by David E. Noakes, T J Parkinson and GCW England, 9th and 10th editions.

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Obstetrical Tools for Handling of Dystocia in Large Animals

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Dystocia, difficult birth, is an emergency that necessitates its prompt handling by a qualified person in order to save the life of calf/dam or both. It is well understood that losses would be enormous in case of loss of either and associated loss/delay in achieving the peak production. Problems at parturition have serious implications on postpartum productive and reproductive efficiency in dairy animals. Usually this period is ignored by the farmers but needs critical care to avoid further complications. Animals suffer from many metabolic derangements and oxidative damage that influences post-treatment convalescence and reduces the dam survival. Other metabolic disorders like ketosis, milk fever at this time also affect the production levels significantly. Increased placental retention, metritis and cystic ovaries are often the sequel to dystocia. A veterinarian can play an important role to minimize them by educating the farmer/dairy man regarding the problem and the time when expert help is required to assist the delivery of the fetus.

Causes of Dystocia

Broadly, the causes can be classified into Maternal or fetal causes. Maternal and fetal causes are immediate causes where the veterinarian is engaged to correct the cause and effect the fetal delivery. Seventy five percent of the total dystocias are due to fetal and twenty five percent due to maternal causes.

Maternal causes

Maternal causes of dystocia may be due to fracture of pelvic bones, small pelvis, congenital hypoplasia of the genital tract, induration of the cervix, peri-vaginal fat, tumours of the genital tract, etc. that reduce the volume of the passage for easy delivery of the fetus. Torsion of uterus is another major maternal cause, esp. in buffaloes, causing dystocia. Other less important causes include ventral hernia, rupture of prepubic tendon, uterine inertia, etc.

Fetal causes

These may be due to abnormal presentation, position and posture of the fetus or oversize of the fetus. During the first or second stage of calving, the extremities of the fetus may get engaged in the pelvic brim or soft tissues of the genital tract, thereby leading to abnormal posture causing dystocia. Other conditions like fetal anasarca, fetal ascites, muscular or pseudo-muscular hypertrophy or fetal malformations (monsters) grossly increase the fetal size causing feto-pelvic disproportion leading to dystocia. A calf weighing 3 kg more than the normal weight usually caused dystocia.

Prediction of dystocia

It is important to predict dystocia in a particular animal to prevent postpartum complications. The correlation between the size of the calf and the size of the maternal pelvis is a conclusive point for the prediction of dystocia. The distance between the tuber coxae (DTC) has been measured for prediction of dystocia. Chances of dystocia were less in animals with DTC of 40 cm or more as compared to animals with DTC of 36 cm. In animals with normal fetal delivery, the ratio of inter-ischial distance and calf fetlock diameter was 3.19 while in animals requiring assistance it was 2.89.

Increase in age, parity and weight leads to increase in pelvic volume and reduction in dystocia. The pelvic canal area was 16 cm smaller in cow heifers having dystocia as compared to animals calving normally.

Diagnosis and management of dystocia

Whenever a case of dystocia is presented, the following stepwise procedure is adopted:

- *History:* History of the case is obtained from the attendant by making cross questions to get the details about the case. Some of the important points include the knowledge about gestation length, onset of labor pains, kind of discharges being voided, any fetal part observed,any abnormal signs shown by the animal, any assistance provided and treatment given to the animal, etc. Knowledge of

these points would assist the vet to diagnose the condition and decide about the kind of procedure to be adopted to deliver the fetus.

- *General examination:* Temperature, respiration and pulse rate of the presented case should be recorded. Observe for any dehydration, condition of muzzle and eyes and flank region for tympany. If the animal is recumbent, dull, depressed and appears to be exhausted, resuscitation is most important before any operative procedure is adopted.
- *Specific examination:* After proper restraint, thoroughly wash the perineal region and do per- vaginal and per-rectal examinations to find the condition of the genitalia and the fetus as such. If there is no uterine torsion, examine for cervical dilatation, condition of the fetus whether living or dead, emphysemated or monster. Particular attention should be paid to presentation, position and posture of the fetus.
- After having determined the status of the fetus and the genital tract, decide to undertake any of the following obstetrical procedures to deliver the fetus. A wise decision save a lot of time and energy and will improve survival of the fetus/dam.
- **Two important steps** before any manipulation in the birth canal include (i) administration of the epidural anesthesia to check straining and (ii) liberal lubrication of the birth canal to facilitate manipulations and safe passage of the fetus. Administration of vegetable oil for lubrication of the birth passage, a common practice in the field, is not recommended due to its harmful effects.

Forced Traction

Generally it is not applied till it is sure that the fetus in the birth canal is presented in a normal orientation i.e. normal presentation, position and posture and the dystocia is due to uterine inertia. It should be done only after proper lubrication of the passage is ensured. Traction should be slow and gentle.

Traction after correction (Mutations)

Traction without correction of the malposture is always dangerous and leads to rupture of the birth canal. Hence, the procedures to correct the malpostures at birth called

mutation should be followed before application of traction to deliver the fetus. These include rotation, repulsion, version and extension of extremities.

Fetotomy

It is defined as per-cutaneous dissection or dismemberment of the fetus to reduce its size to allow easy delivery through the birth canal. It is a technical procedure that requires few careful considerations before it is taken up. It remains the best option available to deliver the obstructed fetus and maintain the future reproductive potential of the animal in serious dystocias due to narrow birth canal, uncorrectable malpresentations and oversized fetus.

Important consideration is that the fetus should be dead, vaginal passage should be sufficiently dilated and lubricated to allow the instruments inside the birth canal. Above all, well designed instruments and technical knowhow to the obstetrician is a must.

Fetotomy could be complete, where maximum of 5-6 cuts are given to deliver the fetus in small parts, or it could be partial where 1-2 cuts are given to deliver the fetus. Most of the cases can be managed through partial fetotomy with high success rate in terms of dam survival and post-operative complications. Following delivery, if the dam feels exhausted, intra-venous fluids, pain killers and steroids be administered along with a course of broad spectrum antibiotics.

Caesarean Section

It is indicated when other obstetrical procedures have either failed or are not feasible to deliver the fetus. Incomplete cervical dilatation, oversized fetus, pelvic deformities and irreducible uterine torsion are the commonest causes for caesarean section. Surgery is normally performed in right lateral recumbency under sedation and local infiltration anesthesia. Right lateral approach is preferred for easy access to the pregnant uterus.

Major post-operative complications of caesarean include peritonitis, adhesions of the uterus with surrounding viscera and wound dehiscence. Moreover, the future fertility of the caesarean operated cases remains guarded due to uterine adhesions.

Post-operative parenteral and intra-peritoneal antibiotics, fluids, anti-inflammatory drugs and local dressing of the wound are mandatory. If performed well within time when the fetus is not putrified, post-operative complications are minimal and animal can maintain fertility.

Uterine infections

The postpartum uterus is highly prone to various insults due to susceptibility following endocrine variations and physical changes at the time of parturition. The convalescence during the postpartum period depends upon the uterine damage during parturition and the inflammatory response. As such the local uterine immunity is significantly lowered due to interplay of hormones and infections gain ground inflicting severe inflammation. A highly significant bacterial load with mixed infection was observed in buffaloes suffering from dystocia. The stress hormones at parturition and during first week of lactation partly contribute to the impaired function of the neutrophils, thereby promoting establishment of the uterine infections. Reduced levels of anti-oxidants and eicosanoid levels at this period also contribute to lowered function of neutrophils. Negative energy and protein balances around parturition also impair neutrophil functions. When compared with the normally calved buffaloes, the phagocytic activity was higher and killing ability of the neutrophils was lower in dystocia affected buffaloes.

Treatment with routine antibiotics does not help and necessitates specific care at this time, otherwise the recovery from infections is delayed and the open period is significantly prolonged. Local administration of immunomodulators like E.coli LPS @ 300 µg, intra-uterine resulted in higher tissue infiltration of polymorphonuclear and mononuclear cells, increased glandular activity and faster tissue repair while changes following antibiotic administration were slow with no tissue repair evident till 48 hours.

This was highly effective to combat the uterine infections and reduce the uterine bacterial load.

It is thus suggested that dystocia should always be considered an emergency and taken up on priority. Any delay of few hours can induce irreversible changes in the tissue and increase already high stress to a limit where the body homeostasis is remarkably influenced.

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Semen Evaluation and Artificial Insemination in Dairy Animals

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Semen: A viscid whitish fluid of the male reproductive tract consisting of sperm suspended in the secretions of accessory sex glands.

Importance of semen examination

- Assessing the fertility of a male animal.
- For breeding soundness evaluation of bulls.
- For semen extension and processing.
- Great diagnostic value in determining the cause, severity and degree of pathological conditions of the testes and other genital organs.

Methods of semen evaluation: Semen evaluation is must before cryopreservation and artificial insemination (AI). On the basis of evaluation criteria, only good semen samples should be processed for cryopreservation and AI purpose. Although semen evaluation alone cannot be interpreted as the sole indicator of the fertility of bull, but it provides significant information on the sexual functions. As soon as semen is received in the laboratory, it is kept in the water bath at 30-35^{o}C till the examination is over. A combination of tests is used to assess the semen quality in an ejaculate as given below:

A. Physical / macroscopic examination

- Color
- Volume
- Consistency / density
- pH
- Presence of foreign material

B. Microscopic examination

- Mass activity / Mass motility
- Individual motility
- Sperm concentration
- Live sperm estimation
- Abnormal sperm estimation

A. Physical / macroscopic examination of semen

1. Color: Normal, freshly ejaculated semen has a creamy to milky white colour. Varying shades of yellow color of semen is normal in some bulls due to riboflavin content which is secreted from ampulla or seminal vesicles. Deviation from normal colour may be due to pathological condition of genital tract. Inflammatory processes impart foetid, foul smell to the semen. The color of semen in cattle/buffalo bull is as follows:

Species	**Color**
Cow bull	Creamy white
Buffalo bull	Milky white

Abnormal color of semen and its possible causes

Abnormal color	**Possible causes**
Yellowish green semen	Infection with *Pseudomonas aerogenosa*
Dark red / pink	Fresh injury to urethra, penis / genital tract
Urine (yellow) colored	Contamination with urine during collection
Pussy	Some suppurative condition of genitalia
Curdy / chunk clots	Inflammation of genitalia

2. Volume: It indicates semen producing capability of testes and accessory sex glands. Age, breed, size, nutrition, exercise and teasing of bull have some relationship with volume of semen production. Volume varies among individuals and between ejaculates within the same individual. Teasing of bulls is practiced to increase the volume of semen. Volume increases with age and body size of animal. Semen volume increases upto 6-8

years of age. Volume varies with general reproductive health, vigour and frequency of service. The volume of semen in cattle/buffalo bull is given below:

Species	**Semen volume (ml)**
Cow bull	3-6
Buffalo bull	2-4

Semen volume will be lower in the following conditions:

- Young males
- Males used excessively
- Incomplete ejaculation or failure of ejaculation
- Bilateral seminal vesiculitis
- Testicular hypoplasia
- Testicular degeneration
- Retrograde ejaculation
- Incomplete ejaculation
- Frequent ejaculation

Semen volume will be higher in the conditions as given below:

- First ejaculate
- Sexually excited properly
- Change of teaser / dummy

3. Consistency and density: It is a rough estimate of sperm concentration. Semen may be thick, thin or watery in consistency depending upon the concentration of sperm. Uniform and opaque appearance of semen is desired as opacity indicates concentration of spermatozoa. The specific gravity of bull semen is 1.036. There is a positive correlation between specific gravity and sperm cell concentration. The score used to express density is 'D'. The appearance and colour of semen has been related to the sperm density. The consistency and density are assessed by slightly tilting the semen collection tube and graded as follows:

Consistency	Density	Concentration	Grading
Thick	DDD	≥900 million	High
Thin	DD	300-600 million	Satisfactory
Watery	D	<300 million	Poor

Grading of semen based on color density:

Color Density	Grade
Creamy	DDDD
Milky	DDD
Thin milky	DD
Translucent and cloudy	D
Watery	0

4. Hydrogen ion concentration (pH): The pH is best measured with a pH meter. For routine AI work special indicator paper having a narrow range (6.0 to 8.5) or indicator dyes can be used. Good quality semen is always slightly acidic. The pH depends upon concentration and activity of the spermatozoa. In diseased conditions, azoospermic / necrospermic samples, excessively used bulls, pathological conditions of testes, epididymis, ampullae and seminal vesicles, incomplete ejaculates and increase in time span after ejaculation, pH shifts towards the alkaline side (7.0 or more). pH of semen in cattle/buffalo bull is given below:

Species	pH
Cow bull	6.6-6.8
Buffalo bull	6.5-6.9

5. Presence of foreign material: Foreign material may enter into semen from animal, environment and/or AV and affect the quality of semen. Presence of foreign material in the fresh semen indicates that the sample is not to be processed further. Following materials may be present inside the semen:

Dung, pus, urine, hair, dust – from animals

Sand, bedding materials, dry dung, insects – from environment

Water, lubricant jelly, dusting powder – from AV

B. Microscopic examination of semen

1. Mass activity / Mass motility: Motility is the most common and extensively used tool for estimating the semen quality. Mass motility is the collective movement of sperm in fresh, unextended semen. Mass motility is also influenced by season. In hot and humid climate, the semen motility is adversely affected. Care should be taken to protect the semen sample from cold shock that markedly depresses sperm motility.

Procedure

- Take a clean, grease free and warm (37ºC) glass slide.
- Put one small drop of neat semen on the slide.
- Keep the slide on biotherm at 37ºC and examine under microscope at 10x magnification.

Interpretation: Slide is observed for presence of waves, swirls and eddies. Assessment could be made on the basis of following observations:

Parameters	**Inference**	**Grade**
Very vigorous forward motion, extremely rapid waves and eddies, about 90-100 % active sperm.	Excellent	++++
Vigorous, progressive movement with rapid and abruptly forming waves and eddies, about 70-80% sperm are motile.	Good	+++
Progressive rapid movement of sperm, slow moving waves and eddies, 50-60% sperm are motile.	Fair	++
Oscillatory or rotary movement, no waves and eddies, 30-40% progressively motile sperm.	Poor	+
Immotile sperm	All dead	0

Evaluation depends upon the experience of the operator. Grade +3 and +4 samples are acceptable. Others should be discarded.

2. Individual motility: It is the movement of individual sperm of extended semen under higher magnification. It is observed to estimate the total percentage of motile sperm in the ejaculate. Exposure to heat, cold, any kind of residue on collection equipment and wrong pH or osmolality of extender can adversely affect motility. Sperm with circular motility indicates cold shock. Various types of motility may be observed:

- Progressive movement: Sperm with very rapid straight forward direction
- Circular movement: Sperm with movement in circular path
- Reverse movement: Sperm moving in reverse manner
- Oscillatory movement: Sperm with jerky movement

Only progressively motile sperm are taken into account while estimating the initial motility.

Procedure

- Take a clean, grease free and warm (37ºC) glass slide.
- Put one small drop of extended semen on the slide and cover it with coverslip.
- Keep the slide on biotherm at 37ºC and examine under microscope at 40x.

Interpretation: Based on progressive motility, the semen samples are graded as follows:

Progressive motility (%)	**Grade**
80-100	Excellent
60-80	Good
40-60	Fair
20-40	Poor
0-20	Very poor

Desirable value: A good semen sample should have an initial motility of 70%. In frozen thawed semen sample, the individual motility should be >40%.

3. Live sperm count: Determination of sperm vitality is an important part of the spermiogram. The live sperm concentration is directly related with motility and the fertility. Differential staining technique has been utilized for counting live and dead

spermatozoa in semen. This is known as "vital staining technique". Eosin has been used as a major cellular stain that stains the cells, whereas Nigrosine stain mixed with the cellular stain has been used as background stains to increase the contrast and visibility / appreciation of the live/dead spermatozoa.

Principle: This technique is based on the penetration of a major cellular stain (Eosin) in dead spermatozoa, whereas an intact membrane does not allow the same stain to enter. Only live sperm participate in fertilization process which is prerequisite to fertilization.

Procedure

- Take 8 drops of stain on a pre-warmed, grease free glass slide.
- Put 1 drop of fresh semen and mix with Eosin-Nigrosine stain.
- Keep the semen-stain mixture at 37ºC for 1 minute.
- Prepare a thin smear of semen-stain mixture.
- Dry the smear in air or on hot plate.
- Examine the slide under 100x magnification (oil immersion)
- Count the stained and partially stained sperms and consider them as dead.
- Count the unstained sperm and consider them as live.
- Count at least 200 sperm from different fields and calculate the percentage of live and dead spermatozoa.

Interpretation: Eosin stains the dead sperm as pink or red. Partially stained sperm are also considered as dead. The sperm which are colorless at the time of staining are considered as live.

Calculations

$$\text{Live sperm (\%)} = \frac{\text{Number of live sperm}}{\text{Total sperm counted}} \times 100$$

4. Sperm abnormality: A normal mammalian spermatozoon under microscope shows two parts viz. head and tail. Any deviation from normal morphological structure of spermatozoa is called as abnormal sperm.

Procedure

- Take 8 drops of Rose Bengal stain on a pre-warmed, grease free glass slide.
- Put 1 drop of fresh semen mix with Rose Bengal stain.
- Keep the semen-stain mixture at 37ºC for 1 minute.
- Prepare a thin smear of semen-stain mixture.
- Dry the smear in air or on hot plate.
- Dip the slide in distilled water so that the excess stain may be removed.
- Examine the slide under microscope (100x) and count the morphologically abnormal sperm.
- Count at least 200 sperms from different field.

Desirable values: In bulls, a good semen sample should not exceed have >20% sperm abnormalities.

Calculations

$$\text{Abnormal sperm (\%)} = \frac{\text{Number of abnormal sperm}}{\text{Total sperm counted}} \times 100$$

5. Sperm concentration: It is required to rule out any pathological conditions in which sperm production is decreased. In fresh semen, sperm concentration is determined to fix dilution rate and in frozen semen samples, *to ensure sufficient concentration is packed in the straws.*

Procedure

- Take 1 drop of semen on a clean glass slide and suck it up to 0.5 mark of RBC counting pipette.
- Clean the tip of RBC counting pipette with tissue paper.
- Suck sperm counting fluid in the same RBC counting pipette up to 101 mark.
- Mix thoroughly semen and sperm counting fluid by rolling RBC counting pipette between palms.
- Take Neubaur's chamber and after moistening its two edges, put one coverslip.

- Release few drops of semen-counting fluid mixture and transfer very small drop of semen between the Neubaur's chamber and coverslip.
- Allow to settle the semen for 2-3 minutes.
- Focus the central squares used for RBC counting and count sperm in 'L' pattern.

Calculations: The concentration is expressed as:

No. of sperm/cc ml = 10000000 x A = 10 X 10^6 x A sperm

Where A is the number of sperm counted

Species	**Sperm concentration (million/ml)**
Cow bull	800-1400
Buffalo bull	600-1200

Artificial insemination: It is the deposition of artificially collected semen in to the female genital tract by artificial means under hygienic conditions.

Thawing: It is the re-warming of frozen semen. The thawing of semen should be rapid. For practical purpose in our weather, thawing of straw is done in a thaw bath at 37ºC for 30 seconds. Hence, it is important that the temperature of the thaw bath is checked immediately before removing the straw from the cryocan. Preferably, use an easy-to-read thermometer.

Advantages

- To have maximum utilization of potential bull.
- To decrease the transfer of venereal diseases.
- To decrease the management cost of bull maintenance.
- Quick genetic improvement.
- Economical (Large number of cows can be inseminated at lower cost in short time).
- Long storage of semen, progeny even after the death of the sire.
- Easy to transport the semen at distant places.

Time of AI

- Insemination should always be preferred at least 10-12 hours before ovulation.
- Optimum time to inseminate a cow is between 6 to 18 hours after onset of estrus (standing estrus).

Check points before insemination

- If the animal to be inseminated is heifer, check its body weight, which should not be less than 240-250 kg in cattle heifers and 300 kg in buffalo heifers at the time of first service.
- There should not be any abnormal discharge from genital organs.
- The animals should not be a problem breeder. Such types of animals should be treated first and then inseminated.
- Proper rectal examination should be done especially for pregnancy before insemination.
- Animal must be thoroughly examined for true heat at the time of insemination.
- There should be a gap of about 60 days after normal calving and of 90 days after abnormal calving like dystocia, abortion, retained placenta etc.
- The type of semen used should be decided in advance.
- If an animal is presented at AI center or in the hospital, the animal should be restrained properly in the crush. If the insemination is to be performed at the doorstep of the farmer, then the animal is restrained with the tree or any best possible facility available with the farmer to restrict the body movements.
- Insemination is always done by recto-vaginal method.

If the above things are not checked prior to insemination, there are chances that the semen may go waste or there may be problems during calving.

Methods of insemination in cattle and buffalo

Recto-vaginal method: This method is also called cervical fixation method.

A. Insemination of frozen-thawed semen

Procedure

- Arrange a clean thawing flask with water at a temperature of 35-37°C.
- Identify and lift the desired canister upto the lower neck (below the frost line) of the liquid nitrogen container and pick the selected semen straw with the pre-cooled forceps.
- Thaw straw horizontally in water at 35-37^{o}C for 30 seconds. During this period the entire straw must be completely submerged in the water bath.
- Wipe the semen straw with tissue paper or absorbent cotton properly.
- Take a clean dry AI gun.
- Hold the gun in your left hand and pull the piston out by about 14-15 cm so as to accommodate the semen straw in the barrel.
- Load the semen straw from its factory seal end and gently tap the barrel of AI gun to move the air bubbles towards the laboratory seal end of semen straw.
- Make a clean horizontal cut through middle of the air space, about 1 cm below the laboratory seal with a clean scissors while keeping the gun parallel to the ground.
- Insert a sterile plastic sheath from its slit end onto the AI gun barrel and slide it down to the collar of the gun and lock it with plastic ring provided with the gun.
- The AI gun is ready for performing insemination.
- Insert lubricated hand in the rectum and after removing dung, grasp cervix and place thumb at os-cervix.
- Get the vulval lips cleaned and opened wide apart.
- Insert the inseminating gun into the vagina at an angle of 45° to avoid the sub-urethral diverticulum and urethral opening and push gently into the vaginal canal carefully.
- Push the cervix forward to straighten the vagina and abolish vaginal folds.
- Manipulate the AI gun gently so as to strike the thumb placed already at cervix and direct through the cervix. Grating sound can be felt while moving the AI gun against the cervix.

- Gently remove your thumb and pass the inseminating gun into the external os and into the cervical canal.
- Deposit the semen gently by pushing the plunger/stillette in the body of the uterus.
- Gently remove the inseminating gun.
- Massage the clitoris at the ventral commissure of vulva.

B. Insemination of chilled semen

Procedure

- Restrain animal properly and clean the perineum.
- Sterilized glass pipette of about 40-42 cm is fitted to a clean and dry glass syringe (2-5 ml capacity) with rubber adaptor.
- After sucking some air (about 2 ml), about 1-1.5 ml of liquid semen is sucked in the pipette.
- Pass the glass pipette through vagina in same way as with the AI gun loaded with frozen semen under hygienic conditions.
- Deposit semen in the mid cervix of the animal.
- Withdraw pipette together with the arm in the rectum.
- Massage the clitoris at the ventral commissure of vulva.

Precautions

- Care should be taken to draw the chilled semen into the catheter from vial.
- Use different syringe for each animal.
- Semen should be discharged from the inseminating catheter at the site of deposition by pushing the plunger of the syringe slowly. If pushed quickly air will be forced through the semen column, leaving 25-50% of semen in the catheter.
- Glass pipette is properly washed, dried, wrapped and sterilized for further use.

Appropriate semen evaluation and handling, proper heat detection and site of semen deposition in the female reproductive tract are critical factors related to achieving a successful AI program.

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Breeding Soundness Evaluation of Bulls

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Breeding soundness evaluation of bull is an important aspect of bull selection due to its relatively quick and economically prudent procedure for screening potential bulls. Breeding soundness evaluation implies a complete evaluation of all factors contributing to normal reproductive potential. The procedure may be adopted:

- At the time of purchase of new bull to be utilized for natural service or semen collection.
- At the time of increase in number of infertility cases in a herd or decreased conception rate.
- As a routine procedure in the sperm stations or semen banks.
- Before the start of breeding season if natural breeding is followed.

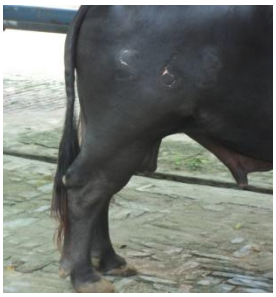
Breeding soundness evaluation involves a through and systemic format for identifying problems affecting male fertility. It involves following steps:

1. Bull identification
2. History
3. Structural soundness
4. Reproductive organs: external and internal
5. Libido
6. Semen quality

1. Bull identification: Bull is identified on the basis of followings:

Date of birth	Special	Body weight
Dam No.	identification mark	Age
Sire No.	Sperm station	Date of
Color	Breed	examination

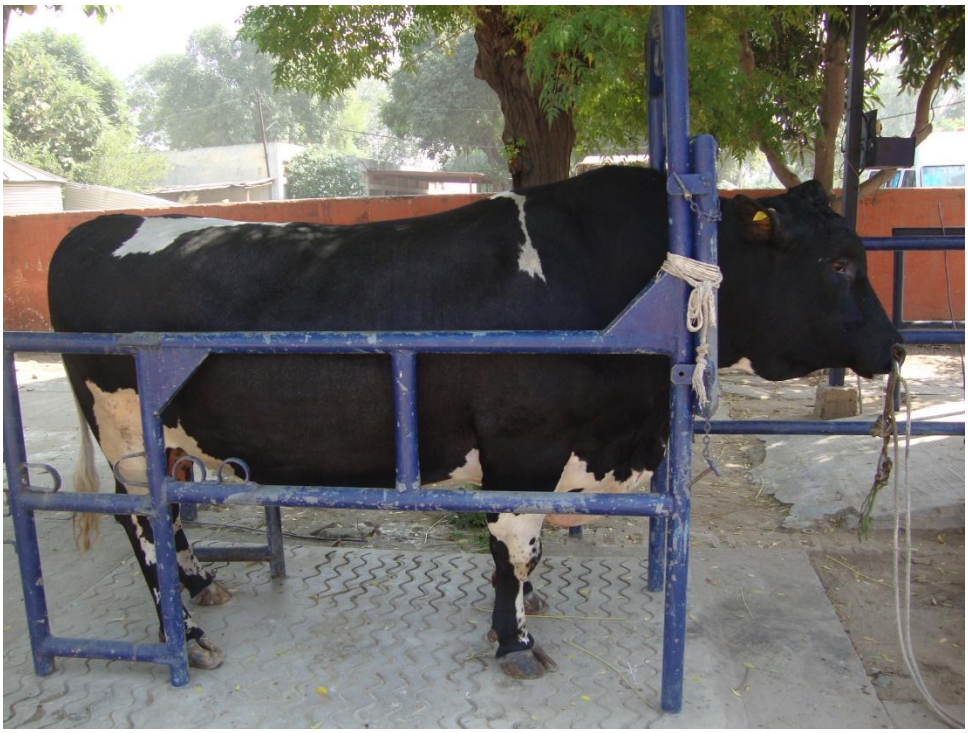
Ear tag



Branding

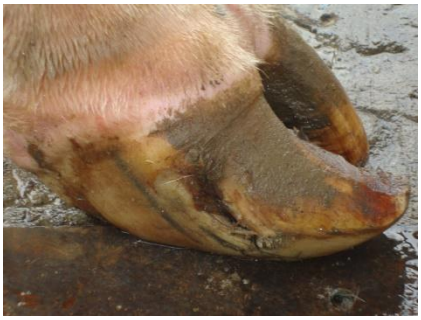
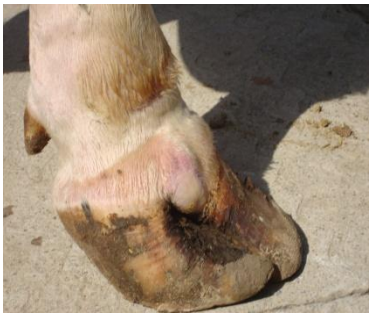
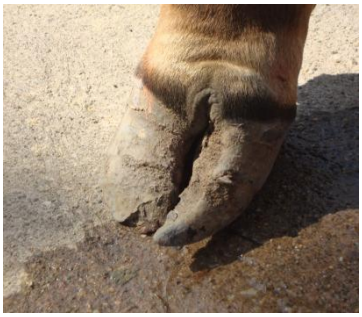
2. **History:** Thorough breeding history of a bull is an important aspect of determining diseases and presence of recessive genes in bulls, which if brought together through mating with other similar genes from a female would create lethal, semi lethal or economically undesirable conditions in offsprings.

3. **Structural soundness:** Before the start of examination of structural fitness of bulls, proper restraining is important to avoid injuries to bull as well as handler.



Structural fitness vis a vis body configuration should be normal to have optimum fertility. Poor health status affects libido, mating ability, semen production and its quality. It includes functional feet, associated joints and overall body confirmation. Any disease

conditions which impair mobility will hinder the performance of the bull. Thorough examination of systems/organs is recommended for breeding soundness evaluation of bulls.

a) **Integument:** Bulls with inguinal, umbilical or other hernias and operative scars may not be recommended for breeding.

b) **Eye:** Eyes should be carefully examined to rule out the possibility of pink eyes, which is indicative of sqaumous cell carcinoma.

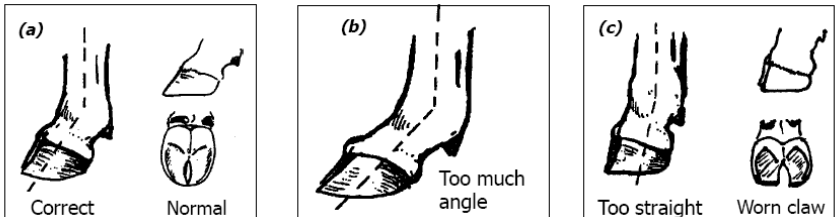
c) **Digestive system:** No abnormality should be in oral cavity and teeth number will provide an idea about age.

d) **Lymphatic system:** During palpation of internal organs, iliac, mesenteric and deep inguinal lymph nodes are easily accessible.

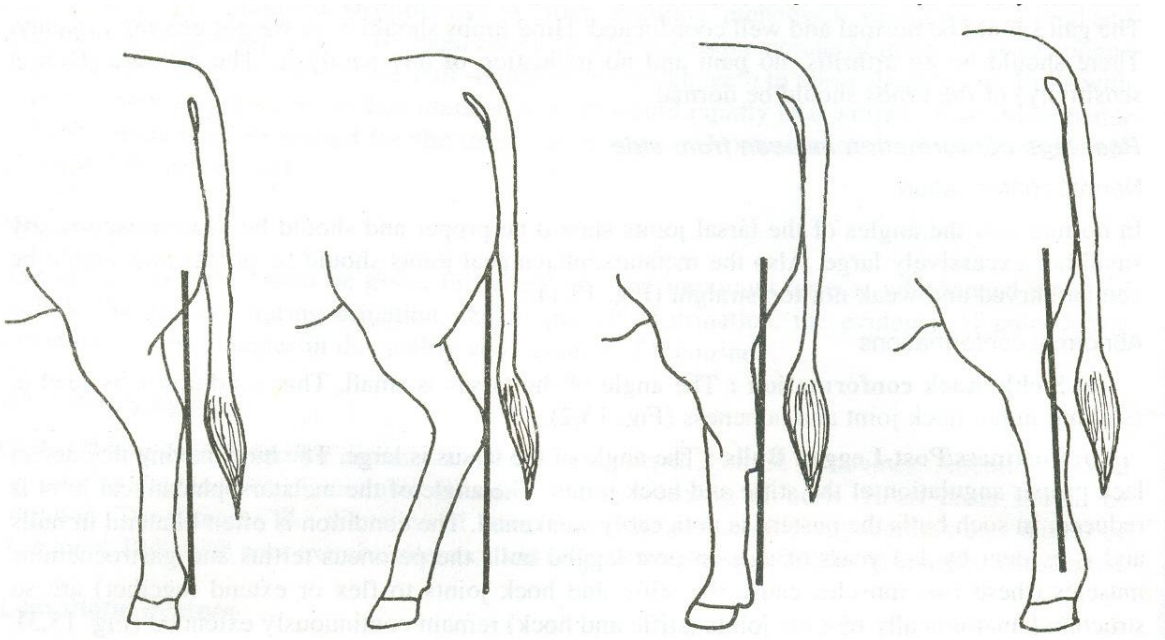
e) **Circulatory system:** Direct palpation of distal aortic, iliac or hypogastric arteries is recommended to rule out any abrasion in pulse.

f) Obesity: Prognosis of structural soundness poor in the bulls suffering from obesity.

g) Fore leges:



h) Rear legs: These should be examined carefully as it bears maximum weight. Hooves should be examined for interdigital fibroma, abscess and overgrown hooves. Following abnormalities may be found in legs:

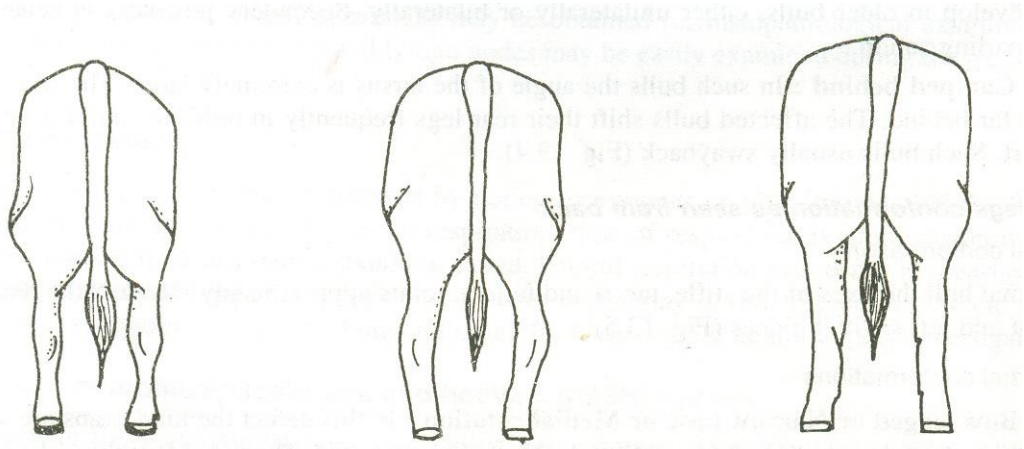


Normal **Sickle hock** **Post legged (Postiness)** **Camped behind**

- **Post legged:** Bulls with this abnormality lack proper angulation of hock and stifle joint. It may be associated with ruptured cruciate ligament and meniscus and pastern noticeably weak.

- **Sickle hock conformation:** This fault may lead to swollen hock and lameness.

- **Camped behind:** Bulls shift their rear legs frequently in an effort to find a comfortable instance. They are usually swayback.



Normal | **Bow legged or Narrow base or Medial rotation** | **Cow hocked or Wide base or Toed out stance**

- **Toed out stance:** It is seen from behind and in conjunction with sickle shaped hock conformation. Wider base is the characteristic in this case.

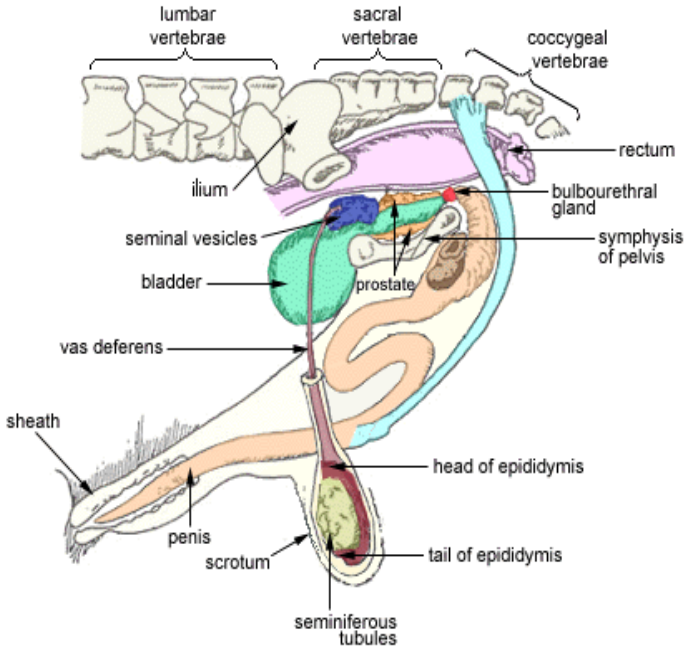
- **Bowleggedness:** The outer margin of hoof is compressed. The outer toe may curl upward, growing over the inside toe and requiring a frequent trimming. Bulls with this fault may show various degree of lameness.

4. Reproductive organs:

a) External: Scrotum, Testes and Epididymis, prepuce penis.

b) Internal: Pelvic urethra, Prostate gland, Seminal vesicle, Ampulla and Inguinal ring.

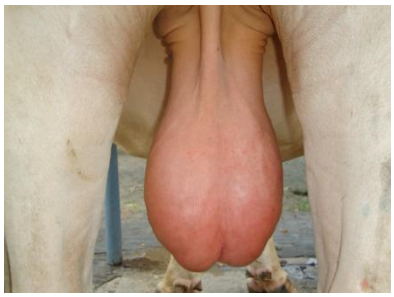
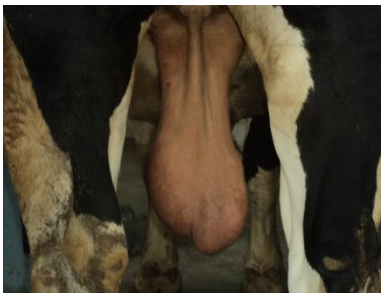
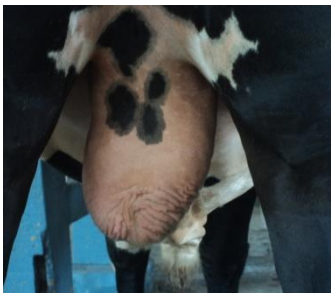
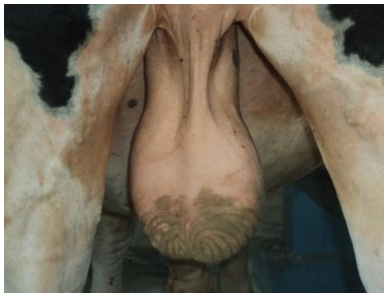
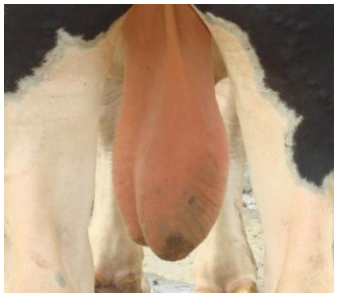
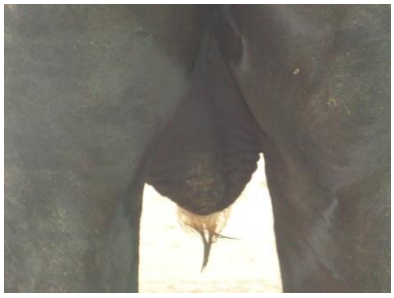
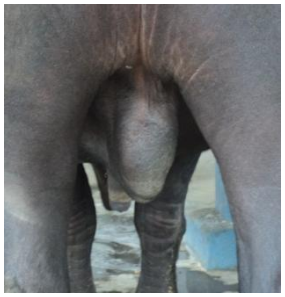
Reproductive organs could be examined properly only after adequate restraining of bull. A strong pole must be placed behind the bull at proper height to prevent kicking. Touching the bull's rump region before examination prevents reaction and kicking.



a) **External**

- *Scrotum:* Scrotal skin should be thin, smooth, pliable and free from ectoparasites. There may be variation in the scrotal conformation. A visual appraisal of the shape of the scrotum in a warm environment, while the bull is relaxed, reveals valuable information about the thermoregulatory abilities of the scrotum. In cooler temperature, the scrotal shape cannot be determined as the dartos muscle in the scrotal wall and the cremester muscles will hold the testicles closer to the body wall. Scrotal shape plays very important role in thermoregulation and is of three types:

- *Normal scrotum:* It is characterized by pendulous shape and a defined neck to the scrotum. Large testicles are most commonly found in a normal shaped scrotum.

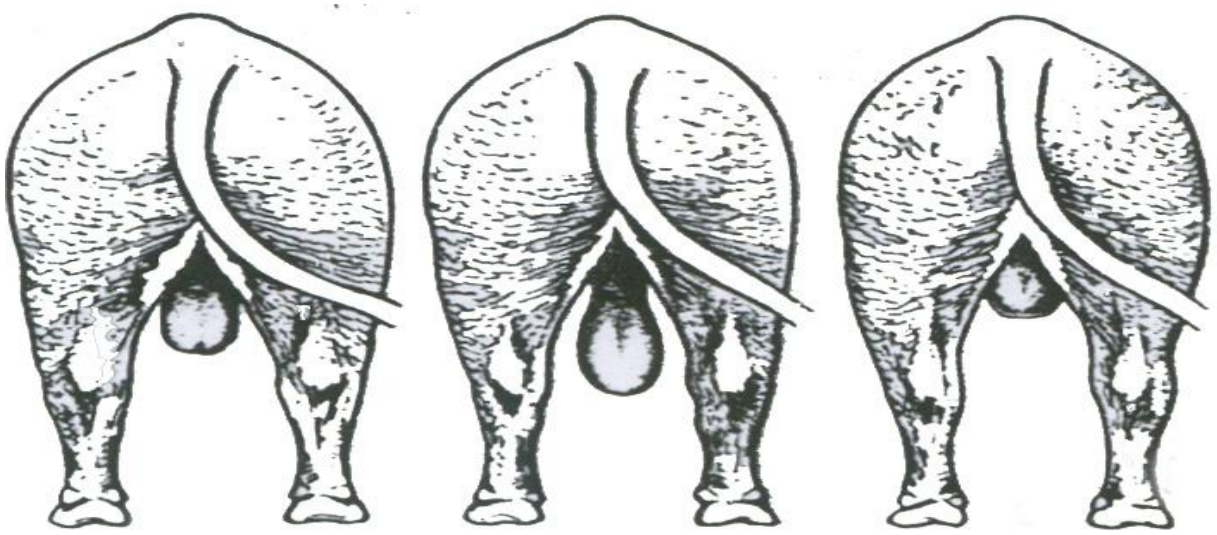








- *Straight sided scrotum***:** This shape is usually due to a combination of small testicles and excess fat in the scrotum. It has compromised thermoregulation and often abnormal sperm production.

- *Wedge shaped scrotum***:** Testicles in wedge shaped scrotum are usually small and held close to the body and contain excessive amount of fat at the neck of the scrotum preventing normal heat exchange. It results in to abnormal spermatogenesis and possibly testicular degeneration.

- *Other abnormal shapes***:** Less commonly, abnormal scrotal shapes may also be found due to short caudal scrotal frenulum, unilateral testicular hypoplasia, orchitis, scrotal hernia, testicular torsion and displacement of the cauda epididymis.

- *Appearance of scrotum from rear:* **The best scrotum is pendulous scrotum with large testes.**

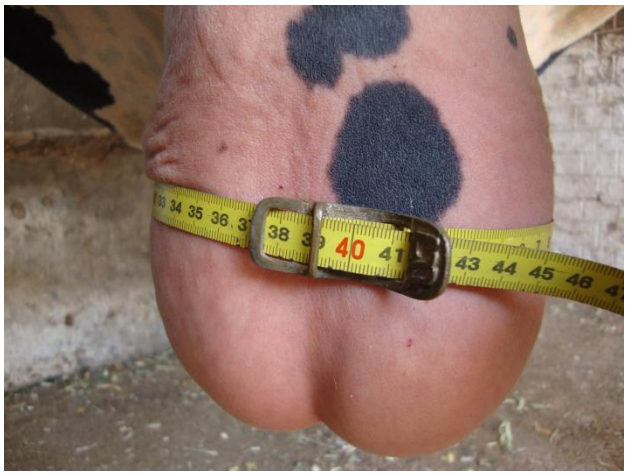


Flat sided scrotum Moderate size testes | **Normal scrotum large testes** | **Ventrally tapered scrotum smaller testes**

- *Trans-scrotal circumference***:** Scrotal circumference is an accurate, repeatable method to access current and future sperm producing ability. It gives an estimate of weight of testes which is directly related to sperm production. Scrotal circumference is also positively correlated with semen volume and quality. Bulls with adequate scrotal development for their age have a higher probability of becoming satisfactory breeders than bulls with smaller scrotal circumference. Scrotal wall thickness, amount of fat in the scrotal neck and lesion on the scrotum must be noted.

- *Techniques of scrotal circumference measurement:* The testicles are palpated and held firmly in to the lower part of the scrotum so that they are side by side to minimize the scrotal wrinkles that may inflate the measurements. However, care should be taken not to force the softer testicles that may compress and expand laterally giving biased result. The fingers or thumb should not be in between the testicles forcing them apart. A looped

measuring tape is kept around the greatest diameter of the scrotum and tape is pulled in such a way that in may be in close contact with the entire circumference. Repeated measurement and its average will reduce the error in the measurement. The average scrotal circumference of pubertal bull is around 32cm.

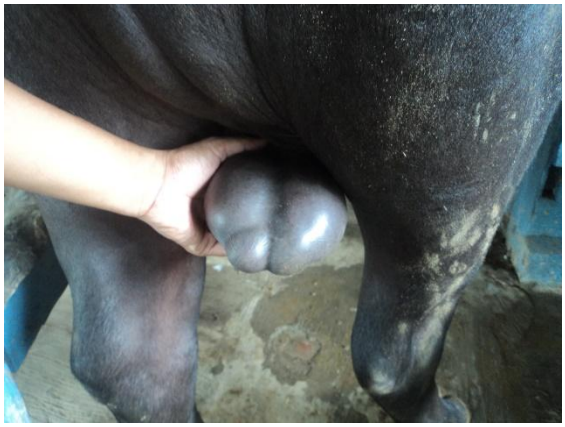


Measurement of scrotal circumference using scrotal tape

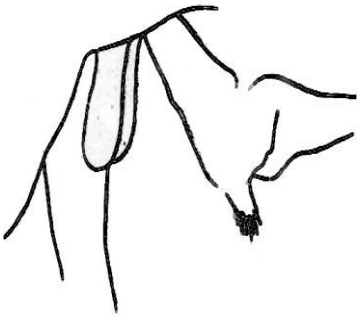
- *Testes:* Testes are located in scrotal sac as too warm temperature is unsuitable for proper sperm production. As the environmental temperature changes, the testes are raised and lowered in scrotum to maintain proper temperature for sperm production. The testicles must be freely movable within the scrotum. While examining the testicle, one testis is moved upward and other is held in the scrotal sac. Testicles should be examined for pathological conditions like testicular hypoplasia, testicular degeneration, Cryptorchidism, orchitis, testicular tumor, abscess, hydrocoel and haematocoel. Any abnormality in testes will render the bull unfit for breeding. The consistency of testis is often difficult to ascertain by subjective method. However, tonometer could be used for this purpose.

- *Epididymis:* The caput epididymis is located craniodorsally on the testis and is usually easily palpable and may be felt more prominent in some bulls. Enlargement in this area may be due to inflammation of sperm granulomas. The body of the epididymis i.e. corpus epididymis can be palpated on the medial aspect of the testicle by sliding the one testicle up. However, it is very difficult to detect abnormality in the corpus epididymis. The

cauda epididymis of a functioning testis is turgid and prominent structure at the base of the testis. Differences in the size and turgidity may indicate the inflammation on one side or may indicate blockade in sperm transport. Segmental aplasia in one or the both epididymis leads to subfertility.



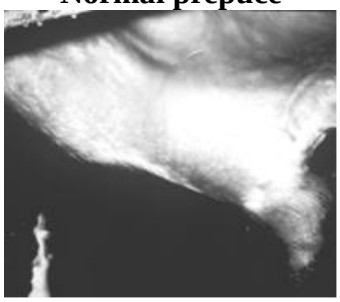
- *Prepuce:* It is examined for any lesions / lacerations / injuries. Pendulous prepuce are more prone to injuries and prolapse



Normal prepuce

Pendulous prepuce

Prepucial prolapse

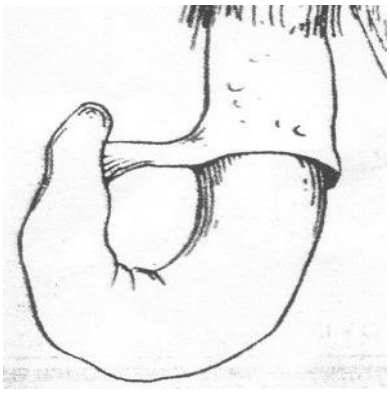


Abscess of prepuce

Prepucial haematoma

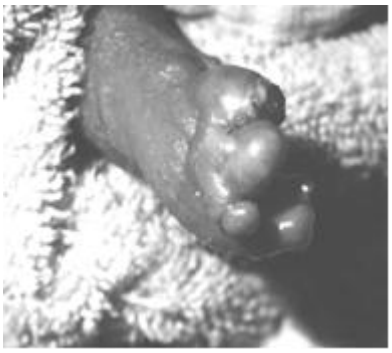
Prepucial stricture

- *Penis:* It is best examined at the time of semen collection. Examined for any physical injuries, lacerations and pathological conditions like fibropapilloma

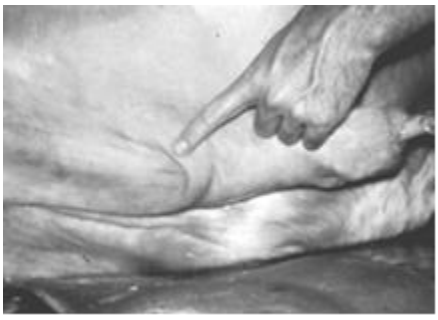


Persistent frenulum

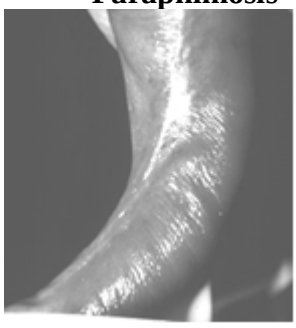
Paraphimosis



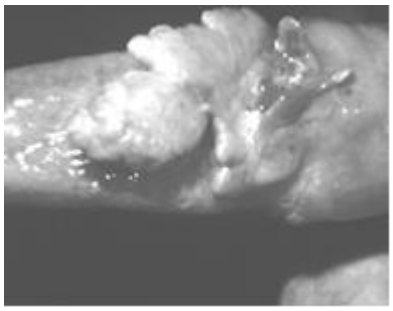
Balanitis due to insect bite



Penile adhesion



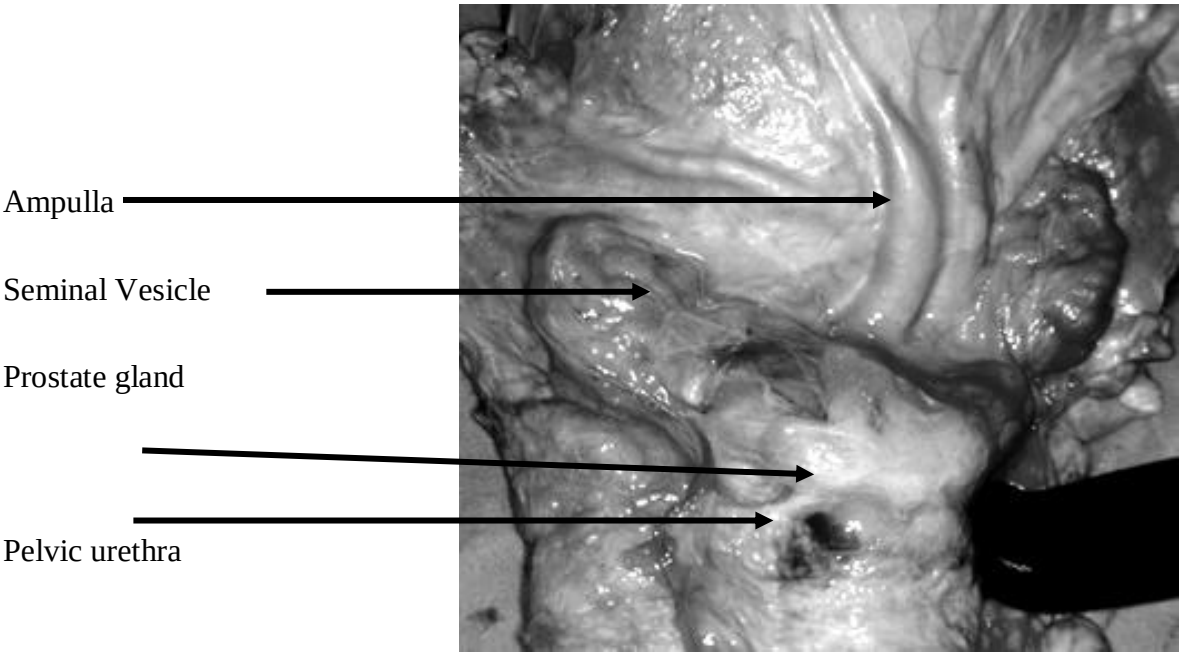
Phyllocampsis



Fibropapillioma

- *Epididymis:* The caput epididymis is located craniodorsally on the testis and is usually easily palpable and may be felt more prominent in some bulls. Enlargement in this area may be due to inflammation of sperm granulomas. The body of the epididymis i.e. corpus epididymis can be palpated on the medial aspect of the testicle by sliding the one testicle up. However, it is very difficult to detect abnormality in the corpus epididymis. The cauda epididymis of a functioning testis is turgid and prominent structure at the base of the testis. Differences in the size and turgidity may indicate the inflammation on one side or may indicate blockade in sperm transport. Segmental aplasia in one or the both epididymis leads to subfertility.

b) **Internal:** The main focus of this examination is on the accessory sex glands and the inguinal rings.



- *Pelvic urethra***:** It is a firm tubular structure which usually becomes pulsatile upon palpation due to the urethral muscle surrounding it. The pelvic urethra is well developed in adult bulls.

- *Prostate gland:* The prostate gland is palpated as a transverse, smooth band surrounding the cranial extremity of the pelvic urethra.

- *Seminal vesicle***:** It is a paired gland and can be palpated cranio-lateral to the prostrate. It is smallaest in the yearling bull and increases with age. It should be uniform, lobulated and movable. The most common abnormality upon per rectal palpation is enlargement, excessive firmness and loss of lobulation. Unilateral seminal vesiculitis is found in brucellosis affected bulls.

- *Ampulla***:** It is 10-15 cm long and 5-8 mm in diameter and may be followed forward to the ductus deferens which leaves the abdomen through the inguinal rings. Pathological conditions in ampulla are not often detected clinically.

- *Inguinal ring:* The internal openings of the inguinal canal can be palpated per rectum 15-20 cm ventral to the pelvic brim and 5-15 cm lateral to the mid line. One of the fingers can usually be inserted in to either opening. In case of large inguinal ring allowing more than three fingers to be inserted, bulls might be predisposed to scrotal hernia. The bulls with large inguinal rings may be discouraged from breeding being heritable.

5. **Libido:** Libido is defined as the willingness and eagerness to mount and attempt service, with mating ability described as the ability to complete service. Deficiencies in either can affect the herd production seriously. High libido bulls are advantageous to herd fertility and have beneficial effects on the fertility of subsequent female progeny. Breeding soundness evaluation for bull is widely accepted but little emphasis is put on libido testing. Libido could be assessed and quantified as follows:

Score	Response
0	No interest in cow
1	Little interest in mounting, sniffing
2	Mounting with hesitation, poor grip
3	Comparatively quick mounting, firm grip and seeking
4	Quick mounting with very good holding and seeking
5	Eager mounting with very good holding and seeking
6	Uncontrolled eager mounting and very good holding and intensive seeking

Maximum score of a bull indicate highest libido.

6. **Semen quality:** The most widely used test of semen quality is the assessment of normal, progressively motile sperm as sperm are the terminal cells which take part in fertilization and whose major role is to carry genome to the oocyte. However, it is a subjective method of semen evaluation and carries quantum of technician's bias. Objective methods of sperm motility analysis include computer assisted semen analysis (CASA), videomicrography, photomicrography and automatic motility analyzer. The advantages with these techniques are determination of percent motile sperm along with distribution profiles of velocity or other kinematic attributes of individual sperm like straight line velocity (VSL), curve line velocity (VCL), average line velocity (VAP), amplitude of

lateral head displacement (ALH), linearity (LIN) and straightness (STR). Other laboratory tests have also been standardized which predicts fertilizing ability of spermatozoa:

i) Hypo-osmotic swelling test
ii) In vitro acrosome reaction test
iii) Cervical mucus penetration test
iv) Oviductal epithelial explant test
v) Zona free hamster penetration test
vi) Competitive fertilization
vii) Sperm zona pellucida binding assay
viii) Hemizona assay
ix) Chromatin analysis
x) Bio-monitoring

The best technique of semen evaluation is test mating with virgin heifer, which is time consuming and costly. So, the above tests may be applied effectively to evaluate the semen.

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Ultrasonography: A Tool to Evaluate Infertility in Male Animals

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Breeding bulls affected with infertility creates problem in meeting the targets of frozen semen production. It has been observed that almost one fourth population of breeding bulls are affected with low semen quality. Various ultrasonographic approaches can be used to demarcate fertile breeding bulls from infertile breeding bulls. Any abnormality in testicular parenchyma can lead to infertility in bulls. Abnormalities in the testicular parenchyma or testicular lesions can be diagnosed via ultrasonography. Ultrasound based evaluation of testicular echotexture can also be used to diagnose infertility. Per rectal examination using 5 MHz linear probe can be used to detect any abnormalities in the accessory sex glands. Screening and selecting bulls for desirable reproductive traits is known to improve the reproductive performance of the herd. So, highly fertile bulls are desired for better herd conception and financial profitability. Hence, evaluation of breeding bulls is imperative for the cost effective production of superior germplasm.

Ultrasonography of external genitalia

Ultrasonography of the external genitalia includes testes, epididymis and pampiniform plexus. Any abnormalities in the testicular parenchyma or testicular lesions can be diagnosed via ultrasonography. Ultrasonographic examinations are performed with a B-mode ultrasound scanner connected to 5.0 MHz linear array transducer. The transducer should be applied transversally on the external face of the testis halfway between the head and tail of the epididymis. Once both testes have been individually assessed, the transducer can then moved longitudinally on both testes. In the centre of ultrasonographic image of testis a hyperechoic area, rete testis is prominent. Hyperechoic images of testes are diagnosed as fibrosis and anechoic images are diagnosed as fluid accumulation in testes. The infertile bulls shows abnormalities like abundance of hyperechoic areas scattered in the testicular parenchyma, acoustic shadowing, showing testicular degenerations with mineralization and many anechoic areas in the testicular parenchyma. Epididymis is seen as fluid filled structure lateral to testicular parenchyma. Any abnormality in the lumen and fluid consistency can be noticed via ultrasonography. Epididymis

can be checked for the normal presence of fluid and any kind of abnormalities like blockade. Pampiniform plexus is seen as network of anechoic vessels and any change in echogenecity is diagnostic of abnormality

Ultrasound based evaluation of testicular echotexture using Image J software

Testicular parenchyma of fertile bulls were found uniformly homogeneous and moderately echogenic. Anechoic and decreased echogenicity of testes were indicative of fluid accumulation and increased echogenicity was indicative of testicular fibrosis. To evaluate the testicular echogenecity with uniformity to represent the echotexture of whole testis, the ultrasonographic image can be divided into 4 squares of 30 mm^2 area. In breeding buffalo bulls decreased echogenecity (mean pixel intensity: 56.29) was due to fluid accumulation and increased echogenecity (mean pixel intensity: 124.81) was due to fibrosis. In crossbred bulls mean pixel intensity was ranged from 23.99 to 124.40 in poor libido bulls and 58.40 to 84.09 in good libido bulls. The narrow range of mean pixel intensity in good libido bulls signifies the homogeneity in echotexture and normality of testicular parenchyma resulting in high fertility. These abnormal conditions might have changed the normal testicular physiology leading to infertility. So, ultrasound based evaluation of testicular echotexture can be used to demarcate infertile bulls.

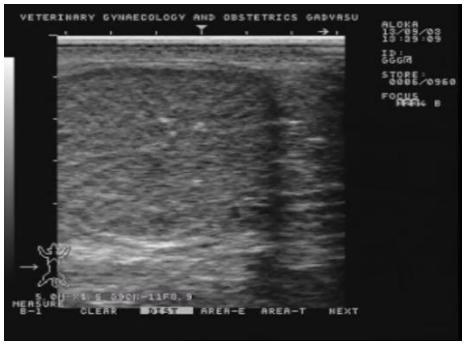
Ultrasonography of the internal genitalia

Per rectal examination should be performed using 5 MHz linear probe to detect any abnormalities in the seminal vesicles and prostate gland. Pelvic urethra is the landmark for the examination of internal genital organs inside the pelvic cavity. Bilateral seminal vesicles can be found on the lateral sides of pelvic urethra. Seminal vesicles can be confirmed by its lobulated appearance. Diameter of seminal vesicles is measured and echogenicity of the fluids present in the accessory sex glands is examined for any abnormalities. Increased diameter and echogencity in the seminal vesicles may be an indicator of seminal vesiculitis. Pelvic urethra should be followed and prostate gland is found as separate fluid filled cavity. Diameter of prostate gland can be measured and abnormal accumulation of fluid can be diagnosed.

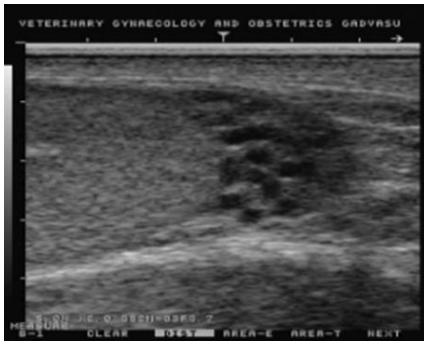
Rump fat thickness

Linear ultrasonographic probe of 5 MHz is successful in demarcation of fat from the skin layers above and muscle layers below fat. Homogeneity of ultrasonographic measurement of rump fat is obtained in the area lying in the halfway between the hook bone and the pin bone. Centre point between hook and pin bone is suitable for placing of ultrasonographic probe. The rump fat layer obtained on ultrasonographic image is usually parallel in the bulls. The subcutaneous fasia is hyperechoic and appeared as thin white layer surrounding the fat from upper side as well as from the lower side. Fat is hypoechoic with low range of contrast. The diameter of rump fat should be measured including the thick fat layer and the two thin subcutaneous fascia layers. High plane of nutrition increases rump fat accumulation. In overfed bulls, there is increase in back fat thickness. Selection of sires with low back fat thickness is expected to have good fertility because testosterone hormone is converted to estrogen in adipose tissue mediated through aromatase enzyme. It is well established that testosterone and estrogen are negatively correlated. So, increased rump fat thickness might be responsible for the problem of infertility and poor libido due to peripheral aromatization of testosterone to estrogen. Proper nutrition is must for maintaining high fertilty in bulls. Routine exercising of breeding bulls should also be performed to prevent excessive accumulation of rump fat.

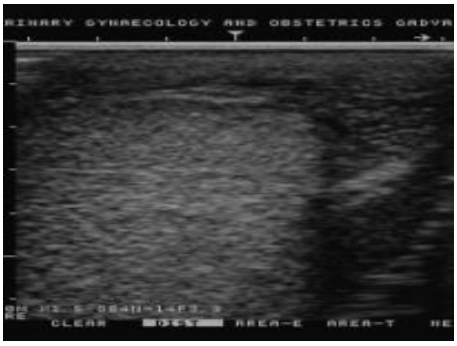
Ultrasonography of external genitalia can efficiently diagnose lesions affecting fertility in breeding bulls. Abnormal fluid accumulation in accessory sex glands can be successfully diagnosed which can cause infertility in breeding bulls. Accumulation of rump fat has an adverse effect leading to infertility in breeding bulls.



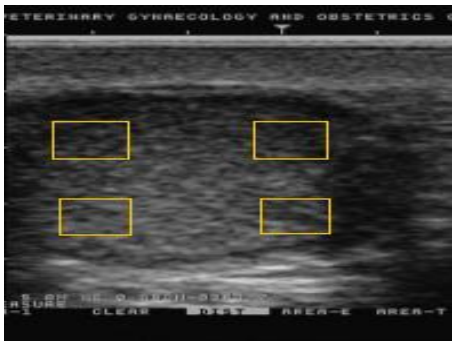
Fibrotic testis



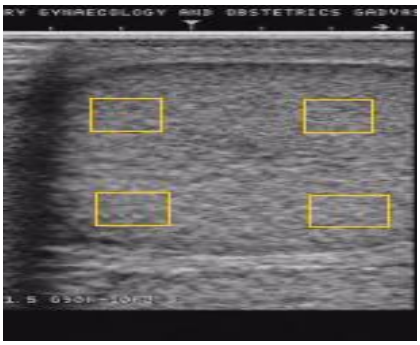
Testis containing fluid



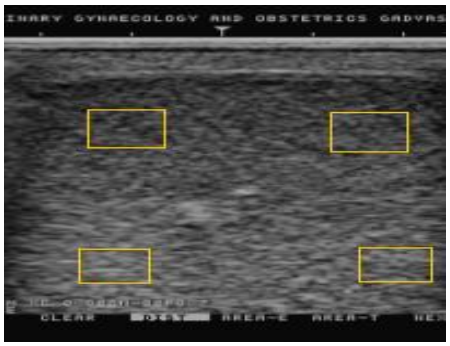
Epididymis



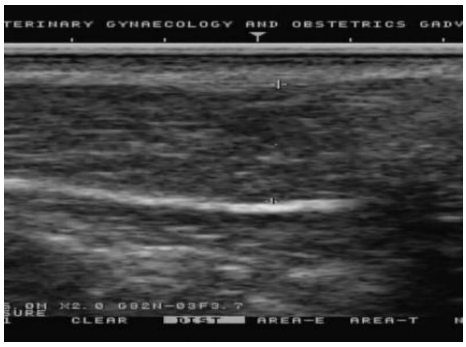
Hypoechoic testis



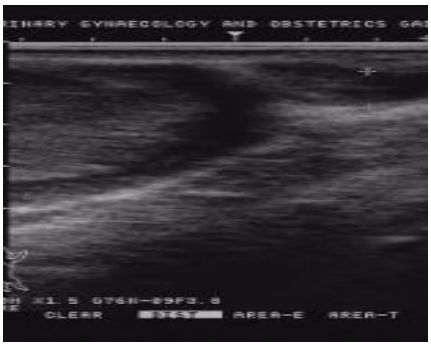
Hyperchoic testis



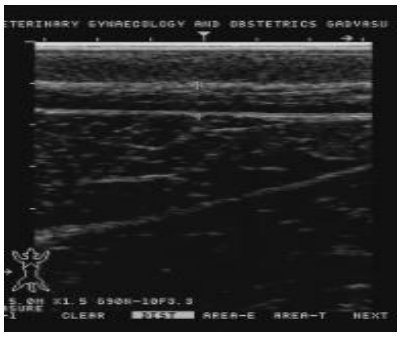
Normal testis



Echogenecity of seminal vesicle fluid



Pelvic urethra and prostate gland



Rump fat thickness (mm); fat layer & S/c fascia layers

Suggested Readings:

1. Kastelic J P and Brito L F C. 2012. Ultrasonography for monitoring reproductive function in the bull. *Reproduction in Domestic Animals* 47: 45-51.
2. Kumar, A., Singh, J., Dhaliwal, G. S. and Singh, P. (2008a). Incidence and factors associated with poor libido in breeding buffalo bulls. *Ind. J. of Ani. Sci.*, 78: 143-45.
3. Kumar, A., Singh, K., Honparkhe, M., Kaur, S., Ghuman, S. P. S. and Brar, P. S. (2016). Physical conformation and ultrasonographic approaches for breeding soundness evaluation of high and low libido crossbred bulls. *Ind. J. Ani. Reprod*., 37:19-22.
4. Singh, K., Kumar, A. and Dadarwal, D. (2015a).Comparison of ultrasonographic testicular echotexture in good and poor libido breeding buffalo bulls. *Ind.vet. J.*, 92: 88-89.
5. Singh, K., Kumar, A. and Honparkhe, M. (2015b).Endocrine status of serum testosterone, estradiol, prolactin and thyroid hormones in good and poor libido breeding buffalo bulls. *Ind. Vet. J.*, 92:39-40.
6. Singh, K., Kumar, A., Honparkhe, M. and Malhotra, P. (2016) Testosterone to estradiol ratio is a differential feature between good and poor libido crossbred bulls. . *Ind. J. Ani. Reprod*.37 (2): 52-53.
7. Singh, K., Kumar, A.,Honparkhe M., Kaur S., Malhotra, P. and Brar, P.S. (2017) Ultrasound based testicular echotexture in realtion to libido in crossbred bulls. *Ind.vet. J.*, 94(04): 14-16

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Handling of Uterine Torsion and Incomplete Cervical Dilatation in Buffaloes

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Uterine torsion is usually defined as the rotation of uterus on its longitudinal axis. Out of domesticated ruminants, dairy buffalo is more susceptible to uterine torsion, as reported in buffalo rearing countries like India, Pakistan and Egypt. The incidence of uterine torsion as well as the time of its occurrence in buffaloes emphasizes its impact on dam's health and thus the dairy herd profitability. Cost-utility analysis of a buffalo with uterine torsion indicated that total loss of Rs 30,000–50,000 for untreated or euthanized animal, mainly due to expenses for the replacement of animal. The loss of a treated animal was around Rs 5,000–10,000, mainly due to loss of calf, reduced milk yield and handling of subsequent conditions, viz. delayed uterine involution, endometritis and infertility. Moreover, torsion of uterus may accompany ovarian vein rupture, rotation of urinary bladder, intestinal obstruction, haemoperitoneum, perforation and formation of adhesions of uterus with surrounding viscera which further deteriorates condition of the dam.

Predisposing factors

Various existing suppositions concerning the maternal and the fetal destabilizing factors liable for the occurrence of uterine torsion in buffalo are unrealistic, however some of these have been justified by logical interpretations. Nevertheless, buffalo reared in open housing system and those reared by nomads in open grazing system rarely encounter uterine torsion. In addition, Indian Murrah buffalo imported by Brazil in 1960s and reared in big pastures on hills, never suffer from uterine torsion. In fact, a study involving 570 buffalo farmers revealed that extensively reared buffaloes were at lower risk of uterine torsion as compared to the stall fed buffaloes. Daily exercise in the form of walk/wallowing reduced the risk of uterine torsion. Keeping buffaloes on kaccha floor is associated with lower risk of uterine torsion. However, segregation of advance pregnant buffaloes and feeding practices had no impact on incidence of uterine torsion**.** This suggests the possibility of poor musculature due to failure of exercise in

buffaloes suffering from uterine torsion as these buffaloes usually belong to farmers who rear buffaloes in closed/tie housing system. Thus, buffalo farming community can be advised to expose buffalo to free movement for some period of the day so that perineal/abdominal muscles become well developed and strong.

- *Changes in uterus:* As the uterus is twisted, the broad ligaments are also stretched and middle uterine vein is compressed. The extent of compression and subsequent damage is proportional to the degree of rotation. Initially there is edema of the uterus followed by hypoxia, ischemia, cyanosis subsequently leading to necrosis of the uterine tissue. This leads to cell death, loss of elasticity and the uterus becomes prone to rupture. Inflammation progresses and there is invasion of uterus by the pathogens. These inflammatory changes subsequently lead to adhesions of uterine wall initially to omentum and subsequently to surrounding organs. Ultimately, delay in correction of uterine torsion causes death of the dam due to generalized bacteremia, endotoxemia or cardiovascular failure.
- *Changes in the cervix:* Cervix is invariably twisted in pre or post cervical torsion. Depending upon the degree and duration of torsion, variable extent of damage occurs to the cervical musculature and its visco-elastic properties are affected. The time lapse between occurrence of torsion and its detection and/or management and degree of rotation is critical. In delayed cases, efforts to achieve effective dilation of cervix usually fail. Even if the cervix is dilated, it becomes prone to rupture. Subsequently, there is delayed cervical involution post-partum or in extreme cases cervical fibrosis may follow which may render the animal infertile or sterile.

Diagnosis

The diagnosis is based on history, clinical signs, vaginal and rectal examinations.

- *History:* The history is critical for deciding the line of treatment. The time lapse between occurrence of torsion and its report to the veterinarian, details of previous treatment and stage of gestation should always be considered. It is important to critically examine changes in the udder, pelvic ligament relaxation, perineal edema and other signs of calving.

- *Clinical signs:* The frequently reported signs are anorexia, frequent straining with lying down and getting up, severe abdominal pain manifested as kicking at belly, tachycardia, tachypnea, decreased rumination, restlessness, dehydration and/or fever in delayed cases. The severity of signs is proportionate to the degree of rotation and duration of torsion. Sometimes clinical signs may not be prominent, if torsion is mild or of less than 180º. The initial manifestation is abdominal pain. All the signs of parturition may be evident if torsion occurs around parturition. There is abdominal straining but no progress to second stage of labor.
- *Per-vaginal / per-rectal examination:* Per-vaginal examination should be done first. Care should be taken to minimize contamination during vaginal examination. The direction of rotation (right or left), location of twist (pre or post cervical) and approximate degree of rotation should be determined. Per-vaginal examination confirms post-cervical uterine torsion whereas rectal examination can establish both pre and post cervical torsion by palpating the altered orientation of broad ligaments or presence of twist on the cervix. A rectal examination is necessary to make a confirmatory diagnosis in case of pre-cervical torsion and to rule out uterine adhesions with adjacent structures.

Treatment

Though many treatment methods have been suggested for management of uterine torsion like vaginal rotation of the fetus, rolling of dam, Schaffer's method or its further modification (Sharma's modified Schaffer's method), laparotomy and caesarean section, the success rates are variable. The clinical evaluation can be effectively used as a tool to decide the appropriate corrective measures. In general, if all the signs of parturition are evident and the animal is presented for treatment early, Schaffer's method (Sharma's modified Schaffer's method) of rolling the dam should invariably be the treatment of choice. The success rates of detorsion after rolling of the dam depend upon the location, degree and the duration of torsion. The number of rolls required to achieve successful detorsion may vary with the degree of rotation. However, it has been suggested that the survival rates are maximum if the number of rolls are limited (less than equal to 3). The method is advantageous as per-vaginal delivery can be affected and there is minimum compromise with post-partum fertility. There should be judicious application of the pressure while rolling, otherwise there are chances of regurgitation and uterine rupture. In

protracted cases the status of udder and ligaments should be carefully monitored. In the delayed cases or in animals with higher degree of rotation, though the detorsion may be achieved, the cervix either fails to dilate or fetal emphysema sets in, necessitating caesarean. The prognosis is not favourable if attempts of detorsion are followed by caesarean section. When the torsion occurs during late gestation (5-8 months), much before the initiation of parturition, though detorsion may be achieved, fetal survival or cervical dilation following induction is questionable. It would be more appropriate to resort to caesarean section under these circumstances.

Impact of duration

An obstetrical case handling in the field by quacks is a major constraint behind the poor survivability of bovines. Depending upon injudicious handing, survival rates of torsion affected bovines presented in <36 h, 36-72 h and >72 h of occurrence of torsion are 52-86, 29-74 and 32-62%, respectively (Ghuman 2010). The success rate for achieving uterine detorsion was higher when the buffalo was presented <36h, whereas the success rate decreased following detorsion of buffalo presented >36h after the occurrence of uterine torsion. As the duration of occurrence of uterine torsion increases beyond 72h, most of the attempts to achieve detorsion of the uterus were unsuccessful. This could be due to development of adhesions between the uterus and the adjoining abdominal organs (Dhaliwal *et al* 1991). In fact, survival rate in torsion affected bovine declines linearly (from 87 to 43%) with an increase in duration of uterine torsion. The duration of uterine torsion and the time taken for complete dilatation of cervix increases the severity of uterine necrosis, fetal putrefaction, maternal toxemia, dehydration, shock and peritonitis. The buffalo that ultimately died following detorsion and the buffalo that delayed the fetal delivery following detorsion had prolonged elevated plasma cortisol as compared to surviving counterparts and the early delivering counterparts, respectively. This warrants creating awareness among farmers and field practitioners' for timely and appropriate handling of an obstetrical case.

Use of Doppler ultrasonography in uterine torsion cases

Doppler ultrasonography-aided assessment of uterine blood flow in relation to duration and degree of uterine torsion was carried out in cattle . Fourteen dairy cattle with uterine torsion were detorted and fetal delivery was completed within 30 min after detorsion. Six live calves

were delivered by cattle having torsion from lesser duration and rest dead calves delivered by dams with prolonged uterine torsion. Whereas the dams of majority of live (n=4/6) or dead (n=5/8) fetus had uterine torsion ≤180° or >180°, respectively. Doppler ultrasonography of middle uterine artery ipsilateral (IpsiUA) and contralateral (ContUA) to the side of torsion was carried out before uterine detorsion for doppler indices viz. blood flow volume (BFV), time-average peak velocity (TAP), resistive index (RI) and pulsatility index (PI). With increase in degree and duration of torsion, BFV in both IpsiUA and ContUA was reduced. In long standing uterine torsion, TAP values were found lower as compared to short duration torsions in both ipsilateral and contralateral uterine arteries. In ipsilateral uterine artery PI (PI-IpsiUA) increased with an increase in duration of torsion. The presence of Pre-diastolic notch in IpsiUA and ContraUA validates the hindrance in blood flow through the vessel and absence of diastole in higher degree and/or duration uterine torsion defined the severity of torsion which further relates to fetal viability. This suggested that assessing the blood flow parameters in middle uterine artery in relation to degree and duration of uterine torsion can serve as useful prognostic indicator. The cattle having lesser degree of uterine torsion could have more chances of fetal survival due to lesser alterations in blood flow.

In uterine torsion affected buffaloes, about one hour before and 30 min after calving, the BFV and TAP values of both MUA were lesser and, RI and PI were higher, as compared to their normal calving counterparts. Subsequently, at 6h after delivery of fetus, the RI and PI values in both MUA of normal as well as uterine torsion affected buffaloes were similar, thus indicating the recovery of blood perfusion to uterus. Furthermore, during post-detorsion period, the impedence (RI and PI) in the MUA decreased leading to an increase in BFV, thus indicating the recovery of blood perfusion. The reappearance of diastole in spectral waveform of MUA after detorsion indicated the recovery of blood flow to the uterus.

Incomplete cervical dilatation

The challenge of achieving complete cervical dilatation in successfully detorted uterine torsion affected buffalo carrying dead fetus can be taken care by cervical massage with Sodium carboxy methylcellulose (SCMC), otherwise leaving the soft or moderately soft cervix on its own to dilate will lead to hardening of cervical texture followed by its failure to dilate. In a

study, a procedure of manual dilatation of cervix was developed for buffalo in which cervical massage for 15 minutes (3 times at hourly interval) can be carried out using warm SCMC. Using this procedure, cervix can be dilated in all the buffalo with soft cervical texture, whereas success rate up to 50% can be achieved in buffalo with moderately soft cervix. Out of buffalo with soft cervical texture and not being subjected to cervical massage, only 29% achieved cervical dilatation whereas none of the buffalo with moderately soft cervix achieved cervical dilatation. In the absence of cervical massage, soft cervical texture was converted to hard texture within 24 h following detorsion of uterus and subsequently cervix failed to dilate. In another study, 24 buffaloes with incomplete dilation after successful detorsion were subjected to different cervical dilation treatments. The complete dilation of cervix occurred in buffaloes (87.5%) treated intracervically with hyaluronidase enzyme, whereas Prostaglandin E1 led to dilation in 62.5% of buffaloes.

Conclusion

Uterine torsion is a serious form of maternal dystocia. The prognosis is favourable if the condition is detected early & managed judiciously. Furthermore, color doppler can play an important role in predicting the prognosis of dam and fetus survivability. Farmers' awareness can help improving calf & dam survival.

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Therapeutics of Retention of Fetal Membranes in Bovines

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Retention of fetal membranes (RFM), or retained placenta, usually is defined as failure to expel fetal membranes within 24 hr after parturition. The definition of RFM is varied; ranging from retention period of 8 to 48 hours postpartum. Therapy is usually instigated during 12 to 24 hours. Majority of cattle will shed the placenta within 6 hours after parturition, with a range of expulsion occurring within 3–8 hr after calf delivery. The incidence in healthy dairy cows is 5%–15%, whereas the incidence in beef cows is lower. The incidence is increased by abortion (particularly with brucellosis or mycotic abortion), dystocia, twin birth, stillbirth, hypocalcaemia, high environmental temperature, advancing age of the cow, premature birth or induction of parturition, placentitis, and nutritional disturbances. Cows with retained fetal membranes are at increased risk of metritis, displaced abomasum, and mastitis.

Retention of fetal membranes is mediated by impaired migration of neutrophils to the placental interface in the periparturient period. The impaired neutrophil function extends into the postpartum period and probably mediates the recognized complications of retained fetal membranes. Cows with retained fetal membranes have increased cortisol and decreased estradiol concentrations in late pregnancy. They may also have an altered prostaglandin (PG) E_2:PGF_2 ratio. Uterine contractility is increased in affected cows. (Placental detachment, rather than uterine motility, is responsible for retention of fetal membranes.)

Patho-physiology of RFM is important to manage a case of RFM. Diagnosis is usually straightforward as degenerating, discoloured, ultimately fetid membranes are seen hanging from the vulva >24 hr after parturition. Occasionally, the retained membranes may remain within the uterus and not be readily apparent, in which case their presence may be signalled by a foul-smelling discharge. In most cases, there are no signs of systemic illness. When systemic signs are seen, they are related to toxaemia. Uncomplicated retention of fetal membranes is unsightly and inconvenient for animal handlers and milkers but generally not directly harmful

to the cow. However, cows with retained fetal membranes are at increased risk of developing metritis, ketosis, mastitis, and even abortion in a subsequent pregnancy. Cows that have once had retained fetal membranes are at increased risk of recurrence at a subsequent parturition.

Manual removal of the retained membranes is not recommended and is potentially harmful. Trimming of excess tissue that is objectionable to animal handlers and contributes to gross contamination of the genital tract is permissible. Untreated cows expel the membranes in 2–11 days. Routine use of intrauterine antimicrobials has not been found to be beneficial and may be detrimental. Although advocated at various times, oxytocin, estradiol, $PGF_{2\alpha}$, and oral calcium preparations have not been shown to hasten expulsion of retained membranes or to prevent complications. When systemic signs of illness are present, systemic treatment with antimicrobials is indicated. In herds in which incidence of retained fetal membranes is unacceptably high, predisposing causes should be sought and eliminated. Supplementation with vitamin E and selenium for herds in which these nutrients are deficient has been found to be beneficial.

Physiology of placental detachment

Cattle have cotyledonary placentas, the fetal cotyledons are attached and envelope the maternal caruncles forming the placentome. Feto-maternal connection is facilitated by, villi from the cotyledons, microvilli interactions at the cotyledon-caruncle interface, collagen links the interface together at several sites and breakdown of this collagen is a key factor in placental separation.

Uterine contractions

Uterine contractions contribute to detachment of the cotyledons from the maternal caruncles – a mechanical process. Lack of damage to fetal villi in normally expelled membranes not purely mechanical. Current thought is that uterine contraction is necessary for the final removal of fetal membranes, primary myometrial dysfunction is not an important prerequisite of RFM. Risk factors associated with RFM are induced parturition, shortened gestation, abortion, twinning, dystocia, fetotomy, cesarean section and nutritional deficiencies such as, Vitamin E,

Selenium and Carotene. Infectious agents that may contribute involve, bovine viral diarrhea virus leading to Immunosuppression.

Etiologies responsible

Mechanisms behind risk factors completely understood, interruption in one or more of these events can lead to placental retention, analysis of risk factors to be done in light of the physiology of placental separation and it helps to determine the various etiologies of bovine RFM. Immuno-suppression in RFM relates to, leukocyte activity, antioxidant capacity, and steroid synthesis. Immuno-suppression in RFM not completely understood. Pregnancy requires immune response suppression to avoid rejection of the fetal-placental unit. RFM might result from failure to switch off these immune-protective mechanisms and because of immune-suppression, or interruption of the normal pre-partum hormonal changes.

Cows with RFM after normal parturition had decreased leukocyte chemotaxis and phagocytic activity before parturition. Neutrophils from cows suffering RFM had decreased chemotaxis from 1 week before to 1 week after parturition and decreased myeloperoxidase activity from 2 weeks before to 2 weeks after parturition. Interleukin-8 is an important chemotactic agent for neutrophils and is seen to be lower in cows with RFM. This suggests a role of decreased neutrophil activity at the placenta may be a part of the mechanism for placental retention.

Decreased antioxidant enzyme capacity of placenta during pregnancy contribute to the etiology of RFM. Cows that subsequently developed RFM had lower prepartum levels of placental superoxide dismutase and lower prepartum plasma estrogen.

Antoxidant enzyme

Overall vitamin E supplementation decreased the incidence of RFM, benefits of supplementation could depend on whether cattle had marginal or adequate serum vitamin E before supplementation. Vitamin E and selenium result in improving antioxidant capacity, increase chemotaxis is and leukocyte numbers at the feto-maternal junction and contribute to the normal expulsion of fetal membranes.

Protease activity differs within placentomes in retained versus non retained placentas, alterations in enzyme activity play a role in the etiology of RFM. Cotyledon collagenase is decreased and type III collagen persists in cows with RFM. Cellular source of collagenases and other proteases in the cow is unknown. Cotyledon or caruncular epithelium and leukocytes are possibilities.

Cows with RFM have decreased activity of MMP-9, cows with RFM lack some forms of MMP-2. MMP enzymes may be important for the breakdown of cotyledon-caruncle links and release of fetal membranes.

RFM mechanism involved

Induction of labor with dexamethasone, with or without prostaglandin, is an established risk factor for RFM in cattle, although the exact mechanism for this is unclear. Suggested proposal is that glucocorticoids could have a direct inhibitory effect on collagenase activity. Dexamethasone inhibits PGF2a synthesis within cotyledon cells. Prostaglandin along with dexamethasone reduces but does not eliminate the occurrence of RFM. Incidence of RFM was reduced when relaxin was administered along with dexamethasone or cloprostenol, relaxin promoting collagenase activity could counteract the inhibitory effects of dexamethasone.

Cows fed anionic diets, those with RFM had significantly lower total plasma calcium that cows without RFM. Calcium is required for collagenase activity. Decreased blood calcium levels found in RFM cows not low enough to preclude collagenase activity. Studies showing comparisons of calcium than cows without RFM. Calcium is required for collagenase activity. Decreased blood calcium levels found in RFM cows not low enough to preclude collagenase activity. Studies showing comparisons of calcium levels between cows with and without RFM have primarily focused on only total calcium than biologically active ionized form. Total calcium levels can be affected by other factors, hypoalbuminemia, in the face of normal ionized calcium levels. Hypocalcemia can predispose cows to dystocia. Uterine atony caused by hypocalcemia can interfere with the final step of placental delivery. Direct role of calcium in placental separation is not clear.

Many risk factors for RFM involve trauma to the uterus. Trauma can result in edema of chorionic villi and impair separation at the cotyledon-caruncle interface. Normal detachment involves separation of the finger-like cotyledon villi from the caruncle crypts. Bigger edematous villi might not be able to disarticulate from the crypts as easily. Trauma to the uterus can cause an increase in heparin release from mast cells at the site of injury. Heparin inhibits collagenases and delays uterine involution. Uterine trauma associated with uterine atony that could inhibit expulsion of membranes and lead to secondary retention. Cesarean section, treatment of cows with a nonsteroidal anti-inflammatory drug (flunixin meglumine) increased the risk of RFM compared with controls. Flunixin meglumine is a cyclo-oxygenase inhibitor and it has been suggested that the higher incidence of RFM is mediated through a reduction of prostaglandin synthesis.

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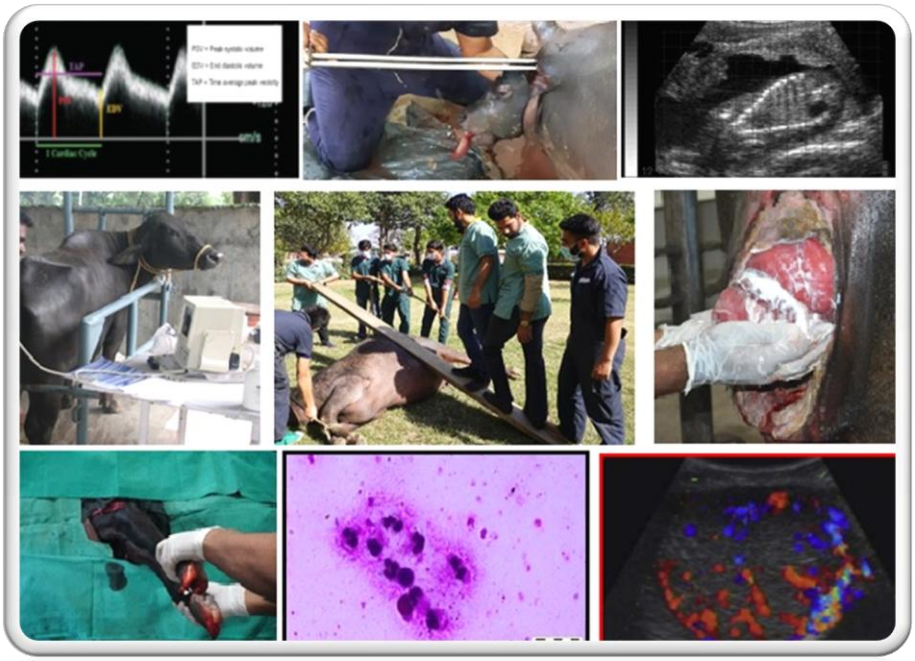

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