



# Proceedings of the 4<sup>th</sup> SIPAR Follow-Up Seminar on Animal Reproduction and Biotechnology for Latin America

February 08-20, 1998





Belém/Castanhal/Pará/BRAZIL





**Editors** Karin Östensson & William G. Vale



# FACTORS AFFECTING FERTILITY OF CATTLE AND BUFFALOES IN TROPICAL ENVIRONMENTS

# Vale<sup>(1)</sup> W. G., BVSc. MSc, PhD, FRVCS, Ohashi<sup>(1)</sup> O. M., BVSc, MSc, PhD, FRVCS, Sousa<sup>(1)</sup>, J. S., BVSc. MSc, Silva<sup>(1)</sup>, A., O. A., BVSc. MSc, Souza<sup>(2)</sup>, H. E. M., BVSc, MSc, PhD and Ribeiro<sup>(1)</sup>, H. F. L., BVSc, MSc, PhD

<sup>(1</sup>Central de Biotecnologia de Reprodução Animal-CEBRAN Centro Agropecuário. Universidade Federal do Pará 66.075-900. Belém. Brazil <sup>(2)</sup>EMBRAPA - Amazônia Oriental, Tv. Enéas Pinheiro, s/n. 66.077-530. Belém. Pará, Brazil Email wmvale@ufpa.br

## Abstract

The present article summarizes the main aspects concerning the reproductive problems, related to milk and beef cattle as well as with buffaloes raised in the tropical areas of the world. Specific aspects which cause a low reproduction due to the genetic or hereditary condition as well as combined to the management and environmental factors like heat stress, affecting mainly European breed originally from temperate climate areas, commonly introduced in the tropical areas, nutritional imbalances, reproductive diseases and other general systemic pathological condition which lead to sub-fertility, infertility or sterility and others constrains, were presented in practical form, pointing out the main problems which routinely, the Veterinary practitioner will find out in the tropical country conditions.

Key words: reproductive problems, cattle, buffalo

# FERTILITY PROBLEMS IN THE FEMALE

# Introduction

The international and national bibliography relates the principal reproductive problem the affects domestic bovine according to an approach that is describe below. by means of classic assignments and in specialized text for bovine (Megale, 1980: Grunert & Berchtold, 1982; Grunert & Gregory, 1984; Vale et al., 1984: Roberts, 1986; Morrow, 1986: Drost & Thomas, 1996: Arthur et al., 1996) and for buffaloes (Vale et al., 1988: Ribeiro & Vale, 1988; Vale & Ohashi, 1994).

# Hereditary problems

# Malformation in the development of the paramesonephrics ducts or Muller

Some alterations in the female tubular genital system that extends from the oviducts to the vulva can take place in the form of abnormal congenitals, that can be manifested by agenesia. hypoplasia, presence of buttons or bands or the tubular lumen aplasia of the uterine horns, stenosis, presence of the median bands giving origin to uterus dydelphus. Specifically in the uterus, they were observed firstly in England by Spriggs (1946) that were denominated White Heifer Diseases, for being diagnosticated firstly in the female of the Shorthorn breed, which was considered a hereditary disease associated to recessive gene that is linked to the sex and white pelage. Later the disease was also diagnosed in Sweden in the Friesian cattle by Nordlund (1956).

#### Chromosome disturbances

Frequently found in bovines *Bos taurus*, both in the beef and milk breeds, that deserve special attention to translocation 1/29 (Robertsonian), described for the first time by Gustavsson & Rockborn (1964) in Sweden. In Bos taurus indicus, is not affected by this abnormality, having being introduced in Brazil through animals imported from Europe and the United States. Due to its high frequency, the clinical significance represents great importance, beside the loss caused by the disease in the animals affected (Pinheiro, 1980). Exist presently different types of structural abnormalities in the chromosomes of the European breeds, distinguishing the 1/18 translocation, in the Swedish cattle, pericentric inversion in the 14 chromosome in the Normand breed, insertion in the 16 chromosome in the Charollaise breed, among others, even though this problem can reach ecotypes originating from crossing between European and Zebus breeds, such as in the case with the Pitangueiras breed and Canchim, in Brazil. It is important to emphasize the presence of these problems in the Bos taurus, needing to have careful attention when importing animals into our country. where carriers of these problems were introduced without any control. In buffaloes. chromosome disturbances are rare, however when crossing occur in the milk breed Murrah or Mediterranean (Bubalus bubalis), whose the chromosome complement is 2n=50, with Carabao breed (Bubalus bubalis var. kerebau), with chromosome complement 2n=48, occur a translocation of the Tandem type due to a fusion between the 4/9 chromosomes, with the products presenting a certain level of sub-fertility.

## Ovarian cysts

By definition, it must be considered as all cystic formations inside the parenchyma or around the ovaries (McEntee, 1990). In specific cases of follicular cysts, exist two peculiar types, that are a microcystic degeneration or a macrocystic degeneration of the ovarian follicles (Grunert & Gregory, 1984). So, the term "ovarian cyst" or "cystic degeneration of the ovary". represent only a morphologic characterization of the presence of one or more vesicles that persist in the ovaries. In specific case of the follicular cyst of the theca and the luteinized follicular cysts, they can be considered as a endocrine disease (hypothalamic/pituitary deficiency), existing however a hereditary predisposition for the appearance of the phenomena, being also associated with high milk production cows, meanwhile, others factors. such as stress and environmental problems, can also be an important component for the establishment of this disease, as can be observed in the following topic (Lopez-Diaz & Bosu. 1992; Woolums & Peter, 1994).

## Irregularities of the estral cycle

Failures in the estrus or heat symptoms, sub-estrus or silent heat, anestrus, irregular or continuos estrus, follicular cyst - nymphomania

## Sub-estrus or silent heat of endrocrine origin

In cows and buffaloes with sub-estrus or silent heat, the ovarian and uterine cycle are present, yet external symptoms of the heat are absent or of little expressivity. According to some authors, the main causes are deficiency of estrogen or progesterone, due to an abnormal sensibilization of the nervous system, would be one of the cause. Hereditary factors, and environmental, and in the last, stress, lack of light, permanent stabulation, high temperatures

# Problems of varied or diverse origins Retained foetal membranes (RFM)

It is the failure in the releasing or expulsion of the foetal membranes within the physiologic period of the understood expulsion in the third stage of gestation or the expulsion phase (Paisley et al., 1986). The RFM are considered if it not expelled 8 hours after parturition. It have great economic importance, especially in milk herds, showing that the economic loss caused by this pathology in a herd of 100 cows with retention rate between 6.6 to 30%, have 40% of the total loss due to a decrease in milk production, 32% in treatment cost, 19% in culling and 9% in the increase of the average interval between parturition (Joosten et al., 1988). The condition has been associated with nutritional factors such vitamin A and E deficiencies, mineral deficiencies or imbalances like Ca, P, Se, I and toxin of nitrate. The main consequences of RFM result in an increased incidence of uterine infection and mastitis.

## Uterine genital catarrh or endometritis

The inflammatory infectious processes of the uterus is denominate acute puerperal metritis, endometritis, metritis and pyometra, Richter (1926), Grunert & Gregory (1984) and Kuntze & Aurich, (1995) and can be classified in:

<u>Endometritis or genital catarrh of the 1<sup>st</sup> Grade - GC 1</u> (*Cervicitis and chronic post - puerperal endometritis*): the female presents normal heats, with varied repetitions, mucous secretion during the estrus with a white-yellowish strings of pus almost unperceptible that can be associated or not with cervicitis, without any evident alterations in the rectal examination. Through the uterine biopsy, a leucocitary infiltration can be found in the endometrium. One of the clinical characteristics more evident are the estrus repetitions in regular intervals, without any apparent cause.

Endometritis or genital catarrh of the  $2^{nd}$  Grade - GCII (*Purulent and catarral endometritis or mucopurulent endometritis*): the female present a greater involvement of the endometrium, due an evident inflammatory process, with a mucous and pus coming from the cervical lumen, that can be associated or not with cervicitis or vaginitis, with the uterus presenting itself flaccid when palpated from the rectum, with normal heats when there is a persistence of the corpus luteum, since in the case of the whole endometrium not affected, exist the possibility that not occur the suppression of PGF2 $\alpha$  production. Due to a purulent secretion that flows through the vagina and vulva, the presence of scabs and humor pasted to the peri-vaginal region and on the tail is a positive symptom.

<u>Endometritis or genital catarrh of the 3<sup>rd</sup> Grade - GCIII</u> (*Purulent endometritis*): the female presents a generalize inflammation of the whole endometrium, with anestrus or long and irregular heats, fetid muco-purulent accumulated in the uterine horns, persistence of the corpus luteum, with the uterine wall being thickened and contracted due to the inflammatory process and the accumulation of purulent secretion.

Endometritis or genital catarrh of the 4<sup>th</sup> Grade - GClV (*Pyometra*): the female presents an accumulation of purulent secretion within the uterine cavity, which can be open or close, that can be considered as a evolution of a genital catarrh of the 3<sup>rd</sup> grade. When closed, no perceptible symptoms occurs through the examination with the vaginal speculum, however, by means of a rectal examination, the findings can be confused with gemelar gestation, due to a asymmetry of the horns and still the corpus luteum is palpable. When opened, the vaginal panorama is similar to the genital catarrh of the 3<sup>rd</sup> grade.

<u>Acute endometritis</u> - (*acute puerperal endometritis*): is the infectious process of the uterus that happens after parturition, also known as septic metritis, with or without the retention of the foetal membranes. In general, a form of inactivity install itself in the uterus, with various germs being responsible for this morbid picture from banal to other pathogenic germs such as *Staphylococcus, Streptococcus, Coliforms, A. pyogenes*, etc. (Grunert & Gregory, 1984; Morrow, 1986).

# Macerated foetus

It is the foetal death, with the decomposition and septic destruction of the soft tissues of the retained foetus, characterized by the softening, disintegration and liquefaction, leaving only the bony tissues, and when palpated there is a cryptation. Occurs in all domestic species, with greater frequency in bovine, due to the entrance of bacteria's in the uterus such as *Actinomyces (Corynebacterium) pyogenes, Staphylococcus aureus, Streptococcus sp., Tritrichomonas foetus*, within others. In the bovine specie, *T. foetus* is the principal etiologic agent involved, whether provoking abortion or retaining the dead foetus in the uterus, cervix or vagina, by the incapacity of the opening of the cervical canal or straightening of the soft foetal route.

The clinical symptoms are anestrus or pregnancy symptoms, even though there may exist straining and contractions that are similar to normal parturition, with vaginal secretion through the vagina, foul smell and sometimes containing decomposed tissues. In the chronic stages, anorexia and toxemia can results due to the absorption of decomposed liquids, with weight loss and endangered the general state of the animal, with a decrease in the milk production of the cow. In some cases, there can be perforation of the uterine wall by bones, with the installation of peritonitis and adherence and the abdominal cavity, that at times can be fatal.

## Mummifiied foetus

Is the modification that the foetus suffers, being dead between the second and the last gestation period and that was not expelled from the uterine cavity. However, it has being found in buffalo, equine, ovine, canine and feline. Even though the causes that leads to the death of the foetus and its later mummification are not yet explained, the following factors must be considered. Only by means of a rectal examination that symptoms are possible to be observe, since the animal apparently don't manifest any. Occur more frequently in bovine between the age of 3 to 6 months, always having a retention of the corpus luteum, the uterus loss its tonus, contract itself in function of the mummified foetal mass, without having any liquid in the uterus and very little placentomas. The cow can continue for 2 to 3 years living normally and through a vaginal examination the cervix is closed with a uterine seal of pregnancy. Infectious agents like C. foetus, Bovine Viral Disease (BVD), uterine torsion, with compression of the umbilical cord, traumatism with foetal death, failure in the postabortion mechanism, genetic and chromosome factors, that contributes to foetal death. The evolution of the mummification can be considered an aseptic process of the involution of the foetus that developed with a closed cervix and with intra-uterine mucous solution without oxygen and without having infection by blood route. According to Grunert & Birgel (1982), this pathologic process is characterized by the following phases: reabsorption of the foetal liquids => reabsorption of the interstitial liquids of the foetus, with consequently drying and shrinkage (volume decrease) drying and adherence of the placenta to the uterine wall, with the

disappearance of the placentomas and the placenta becoming dark, with the foetus acquiring a firm consistence, being called by this the petrified foetus or litopedium, even though it is rare for mummified foetus to presents calcium deposits. According to Long (1996), exist two types of mummification's in bovines that are distinguish by the foetal membranes. The *papyracea* that presents both the foetus and the membranes in dry form, firm, similar to a papyrus and the *hematic*, that even presents the foetus similar to the former, between the uterus wall and the rest of the foetal membranes, there exist a substance similar to that of "coffee colored pap/chocolated", sticky, probably of bloody origin.

#### Mucometra and hydrometra

Is the accumulation of mucous or watery secretion within the uterine cavity. The cow is affected, that can have origin with hereditary disturbances as in the case of the alterations in the development of the paramesonephric ducts or Muller or acquired in the case of cystic hyperplasia of the endometrium, generally associated with cysts or tumor in the ovine, as well as by lesions, scars or adherence in the cervix or in the vagina. The mucous or liquid accumulation due to constant stimulation of the endometrial glands cause an endometrium atrophy (Roberts, 1986; McEntee, 1990).

#### Failure in the development of the endometrial glands

Is the total or partial absence of the endometrium, but very rare in different species. In the total case, the animal presents anestrus, due to the non production of the luteolitic factor -  $PGF_2 \alpha$ , while in the partial case, the cycles can be normal, even though the animal is sub-fertile (McEntee, 1990).

#### **Ovarian tumors**

The cow's principal tumor of the ovary is the granulosa cell tumor (GCT) and of the buffalo is the teratoma, even though the teratoma also occurs with a certain frequency in the taurine and zebu's cow (Vale et al.,1984). Generally the GCT have endocrine function, with abnormal proliferation of the thecas and granulous cells, that can likely, produce both estrogens as well as progesterone. There can be alteration of the estral cycle in this case, with nymphomania or anestrus. With respect to uni or bi-lateral teratoma, the animal can develop a normal pregnancy.

#### Phytoestrogens ingestion

This is associated with the ingestion of estrogenic plants in bovines, especially in pastures with subterranean clover - *Trifolium californium*, that contains isoflavines

(phytoestrogens), that provokes progressive, accumulative and irreversible lesions (Bennetts et. al., 1940). In most cases, a cystic hyperplasia of the endometrium, with muco or hydrometra, and sterility of the animal is establish.

## Genital infectious diseases

Various diseases can affect specifically the reproduction of beef and milk herds, occasioned from premature embryonic death as well as abortions. Within these diseases we can count brucellosis, that produce abortion between the sixth to eighth month of gestation, being one of the enzootic disease transmitted to humans. The heifers are vaccinated between 6 to 9 months of age, with vaccines proceeding from the cultures of live strain of the  $B_{19}$  type, is the

control method for this disease. Vibrosis or genital campylobacteriosis, caused by the gram positive bacteria Campylobacter foetus, is another disease of great importance, especially in milk herds, provoking embryonic death between the fourth and fifth week, with abortion occurring in the fifth month of gestation. This disease is widely spread, that can attack both the male and female, that is being transmitted by infected bulls. The utilization of AI, is the recommended method for the eradication of this disease in endemic areas. The IPV/IBR, that in the genital form is known as Infectious Pustular Vulvovaginitis and in the respiratory form as Infectious Bovine Rhinotraquitis. is a herpes virus, transmitted both by the genital and respiratory route, also responsible for fertility depression and great economic loss. Bovine genital trichomonisis, caused by the protozoan *Tritrichomonas fetus*, is responsible for the death, maceration of the foetus,

or abortion between the second and fourth months of gestation (Grunert & Gregory, 1984).

# Chronic and debilitating diseases

Every infecto-contagious diseases, such as foot and mouth disease, babesia, anaplasmosis, metabolic diseases and disturbance of the apparatus, as well as systemic disturbances, are also responsible for infertility problems. The cachexia, as well as general debility, accompanied by the loss of the ovarian function in old animals, leads to suppression of fertility, beside being directly related to the economic loss of production of the animals affected.

# Disturbances of environmental and nutritional origin Climatic alterations

The problems linked to temperatures extremes, very hot or cold affects directly the reproduction of lactating females. The thermal stress occasioned by high temperatures, are responsible for the liberation of a series of endogenous factors and substances that signal the hypothalamic-pituitary axle, just like how the Central Nervous System (CNS), causing the animal to react with a decrease of the feed intake, alteration in the hormonal function, drop in the heat production, resulting in the drop or even suppression of the lactation and fertility reduction - anestrus. In tropical climates, when the heat production of the animal exceed the heat loss due to high temperature and ambient humidity, the animal have difficulty to dissipate the heat, increasing a hyperthermia, with a decrease in the feed ingestion, and less quantity of metabolize energy, refusing to eat forage, when to have a compensation. begins digesting diets rich in concentrates, with alteration in the ruminal flora, loss of weight and infertility (Johnson, 1991).

According to Shafie (1994), buffaloes reacts differently with rigid tropical climates, by having a better capacity to adapt in hot and humid areas, due to having superior morphologic and anatomic characteristics than that of taurine and zebus in the fact that a more thicker epidermis that cover its skin, that have a higher number of melanin particles, thus a dark skin. This melanin richness, cause the ultraviolet rays, to be neutralized and reflected, preventing the same to penetrate the inferior derm and consequently accumulating in the organism (Shafie, 1985). Thus, in flooded areas, even when the temperature and humidity are high, the buffalo is superior with respect to other bovines in relation to ambient stress.

## Low nutritional levels

With the deficiency of energy or protein, the reproductive function is one of the first to resent when face with adverse situations related to the nutritional state. Thus, this aspect relates negatively over the general metabolism, installing an anestrus process, with the loss of the immunologic defense mechanisms of the animal. For a precise evaluation of this disturbance, the utilization of the Body Score Condition (BSC) is one aspect that must be considered with great importance, especially when selecting animals to be utilized in controlled reproduction programs (Lotthammer, 1991).

## Excess of nutrients

With the excess of energy and protein the animal has a tendency to obesity, which cause a fatty infiltration in the peri-ovarian region, that can interfere in the ovulation. The same way, excess feeding leads to a delay in the uterine involution, with increase percentage of endometritis, and the appearance of follicular cysts.

## Deficiency and excess of minerals

The minerals deficiencies are recognize as responsible for a series of reproductive disturbances. The sub-clinic deficiency of phosphorus leads to a prolong anestrus, with ovarian atrophy, silent heats and delay in the appearance of the first heat of the post-parturition period. Manganese deficiency with the appearance of anaphrodisia and abortions, while selenium and copper deficiency increase the incidence of RFM, the same way copper also leads to a ovarian atrophy and retardment in the appearance of the first heat of the post-parturition period. Another aspect that leads to reproductive disturbances, is an excess of a determined mineral inhibiting the absorption of the other. This phenomenon called mineralosys, is frequently observed in areas where there exist an excess of aluminum and iron, that inhibits the normal absorption of other essential minerals such as copper, zinc or cobalt.

#### Management errors Lactatioanl anestrus

In all bovidea females, during the post parturition and lactation it becomes critical for the appearance of the heat and ovulation. However, the lactation have considerable effect over the beginning of the ovarian activity, with a milking or nursing frequency influencing the establishment of an anestrus period in the post parturition period in milk producing animals. Successive nursing - young calf, is a limiting factor in the establishment of the lactation anestrus in the cow and buffalo. This aspect is also directly related to the feeding level of the animal. (Vale, 1994).

## Unobserved estrus or heat

Even in normal females, many times the heat can pass unobserved or not to be observed in the case of AI. In extensive management, where is impose a bull/abnormal female relation, it is common many times to encounter cases where five females are in heat at the same period, with some not being able to be served, and consequently a delay in the service period.

## Failure in the pregnancy diagnostic

After being served or insemianted, the female bovine or buffalo must be confirmed pregnant or not, which must be done within 40 days of gestation. Early examinations by means of the radioimmunoassay technique (RIA) to detect the progesterone levels in the plasma or in the milk have being utilized precociously, thus 21 days after service or insemination, and can tell

See WY

according to the progesterone levels if the animal is in gestation or not, that is followed by a confirmation of the rectal examination, after 45 days of pregnancy.

# The repeat breed cow Economic aspects

According to data from DHIA/USA, a cow that repeats three cycle in one interval between parturition's, cause an economic loss of 8.8% in its annual production, that can be understood as 0.15% of a calf born, and 144 Kg of milk for affected cow.

## Anatomic deformities in the ovaries or in the genital tubular system

Anatomic deformities in the female tubular system, both of hereditary or acquired origins, affecting both the gonads as well as the genital tubular system, leads to problems of heat repetition. These aspects were already considered.

# Failure in the conception due to miscellaneous problems

Problems linked to maternal factors, Figure 1, such as endocrine and immunological dysfunction, MRP (*Maternal Recognition of Pregnancy*) failure, asynchrony between



Figure 1. Failure in the conception due to miscellaneous problems, Albihn (1991)

embryo and mother, or embryonic factors, genetic abnormalities, over- matured gametes or improper MRP signal, or factors causing fertilization failure due to low fertile semen or ovulation failure, gamete transport disturbances, reproductive tract malformation, as well as environmental and management factors, that can give origin to unbalanced products, which can not develop normal, die in the uterus and are absorbed or suffers abortion.

# PRINCIPAL PROBLEMS IN THE USE OF ARTIFICIAL INSEMINATION (AI)

The last two decades, a large inputs was reached by bovine breeds due to the utilization of the AI practice. Countries with tropical climate such as Australia. Brazil. Kenya, India Jamaica. Pakistan. Thailand, were able, to use this biotechnique, to establish beef and milk herd originating from the crossing of native breeds with high grade *Bos taurus indicus*, crossed with European milk breeds *Bos taurus taurus*.

#### Management errors

As in the cases with the natural mount, the management errors are also frequent when the AI is utilized, with the same having a higher frequency, due to greater intensification of the production system, which when neglected leads to greater losses.

#### Detection of the estrus

The failure or deficiency in the detection of heat, is one of the principal problems found within the AI program, having by this reason a necessity to concentrate more effort in the sense that improvement of the detection techniques of the heat is needed constantly, whether by conventional methods, such as rectal examination or the utilization of teaser bulls or the use of ultra-sound examination, progesterone levels in the plasma or in the milk. Personal education involved in the AI program, concerning

with the reproductive management, seeking to identify animals in heat, cover a greater importance for the success of any program.

#### Insemination moment

Classically the AI time in bovine was determined a long time ago, by the utilization of TRIBERG'S model or in other words, cows with heat in the morning should be inseminated in the evening of the same day, and those with heat in the evening should be inseminated the following morning. With this model, results of 95% births are obtained. For buffaloes, contrary to what many expert preconized, this model can also work, meanwhile due to intrinsic problems of the specie, many times the animal presents shorter or longer heat, of which it is possible to establish a better model to inseminate the female after the heat symptoms ceases. In many countries where buffaloes have economic importance, this standard have being followed, and in Brazilian conditions, it looks like the best recommended (Vale et. al., 1991; Vale, 1994).

#### Sanitary and hygienic aspects

The AI practice such as the biotechniques required by the Veterinarian and its team, a greater responsibility concerning hygiene. From the equipment's utilized in the technologic processing of the semen in AI centers, until the preparation of the semen during the insemiantion act should follow a rigorous hygiene ritual. Hygiene beforehand of the female to be inseminated, is a basic fundamental requirement to obtain high fertility rates. Should bear in mind also that the female genital system, by having a bacteria flora, is an adverse aspect for the semen to be inoculated in the genital system. The progress obtained in the development of the AI universal gun type, with a double layer protector, having reduced consubstantially the contamination possibility of the semen by means of the female flora to be inseminated.

## Semen quality

The quality of the semen according to the standards of the Ministry of Agriculture must be taken in consideration. Thus, the adopted standards for the commerce of semen and embryos in the MERCOSUL (Common Market of the South American Cone), must be adopted to satisfy not only the regional market, but internationally as well (R.B.R.A., 1996).

## The artificial insemination technique correctly speaking

The AI in bovines and buffaloes must be the deep cervical type, or in other words in the body of the uterus. Deposition of the semen in the cervical canal, is the principal cause for low conception rate, being the main discouraging factor for inseminators or farmers who are beginning the use of this biotechnique. The deposition of the semen in the uterine horn upsilateral or counter-lateral of the ovary that ovulate. don't causes low conception rate (Mamont et al., 1989). Various studies have demonstrated that the correct deposition of the semen is one of the positive points for the success of the AI in these species, and one of the causes of this problem is the inadequate preparation of the inseminators and failure in the selection of female for the AI programs (Senger et al., 1988). Thus, the recycling and professionalization of the inseminator is of great importance to obtain more favorable rates.

## Management problems of the frozen semen

Adequate conservation of the semen in a container with liquid  $N_2$  and its normal storage capacity, is one of the important factors for the success of the AI, in the tropical areas.

# FERTILITY PROBLEMS IN BULLS

## Introduction

Many times fertility problems of the bovine and buffaloes herds can be related to the male. Hence, it is also necessary to take in consideration the routine examination of the male, establishing a diagnostic of its health and hereditary, because many infertility and sterility problems in the male have hereditary base. Thus the fertility of a bull plays a fundamental economic role in the herd. (Blockey 1976a-b;Galloway 1976). For those authors - "<u>a fertile</u> bull is that with the capacity to generate offspring's, when in contact with 50 females during six weeks, is capable to fecundate 95 -100% of them, with most of them conceiving in the first three weeks". Meanwhile, when a disturbance is installed that affects the reproductive capacity of the bull, an infertility or sterility picture is being installed that can have an alteration of the sperm picture of the ejaculate. However, the fertility of the bull depends on the percentage of the normal spermatic cells being ejaculated, because a high number of abnormal sperm cells decrease the fertility of the bull, being of great the importance the andrological examination, to know the fertility of the bull, especially its seminal picture.

## Clinical examination of the genital system of the bull

This procedure is equivalent for all males of the different domestic species, having in mind the specific characteristics for each one (Lagerlöf, 1951, Galloway 1976, Merkt & Krause, 1977). Thus, the <u>andrological\* examination</u> consist the *EXAMINATION* and the *JUDGEMENT* of the examined male, whose objective is to reach a diagnosis, taking into

consideration its *HEALTH* and *SEXUAL FUNCTION* - <u>sexual behavior</u>\*\* through the following criterion's:

1. **HEREDITARY HEALTH** (the animal must be free from diseases or hereditary deformities, that restrict the use as a sire).

II. GENERAL HEALTH ( the animal must be free from diseases extra-genitals that limits its utilization as sire).

Ill. **NORMALITY OF THE GENITAL SYSTEM OR SEXUAL HEALTH** (must be free principally of functional disturbances and infections).

IV. COITUS CAPACITY - "*POTENTIA COEUNDI*" (to be capable to have a complete coitus and without interruptions).

V. **FECUNDATING CAPACITY** - "*POTENTLA GENERANDI*" (capacity to fertilize, by means of a normal quality of the semen - normospermia).

## **Examination procedures**

Species, breed, age. Identification characteristics (tattoo, ring, brand, pelage color, etc.).Type - masculine or feminine.

#### History and annamnese

The management and feeding program of the male can be of great importance, especially when handling animals that are imported from temperate or sub-tropical regions to tropical climate regions, which is of great interest when European bovine breed are imported from temperate climate. The history of the previous reproductive performance and its efficiency as reproducer, as well as progeny quality, reveal as the fundamental interest of the clinical reproductive point of view. The number of females with which the male was mated, (non return rates) in 30, 60 and 90 days, for bovines and buffaloes.

## **General examination**

A general clinical examination of the different systems, which will not be describe here. During the clinical examination of the male. it is very important a minute observation of the animal walking, thus it would be possible to discover the presence of lesions in the vertebral column (spondilo-artritis, vertebral osteitis dorso - lombar) that can limits coitus due to the pain, leading the animal to present an impotency capacity. To observe specifically the hind, principally the deformities of the vertical position, lameness, spasm syndrome, scars arising from lesions or treatments. Disturbances of the skeleton muscles system and locomotor that affects fertility. Great importance has the presence of the spasm paresis or syndrome in bovines, whose probable cause is an alteration function of the CNS, of hereditary origin, which presents itself in uni or bi-lateral form in the posterior extremities, characterized by a rectilinear extension of the limb.

<sup>\*</sup> Andrology: textually mean "science of the man or of the masculine sex", including all masculine problems, in biologic, pathologic and therapeutic sense.

<sup>\*\*</sup> Sexual behavior: means the sexual performance of the animal as a sire, including phenotype, descendent, productivity characteristics in general.

# Special clinical examination of the masculine genital system

The objective of the examination is to determine: the morphologic aspects in the different segments; the morphology and size in relation to age and body development of the animal, to detect the existence of possible abnormalities.

## Clinical examination of the external genital organs Prepuce and penis

Both bovine and buffalo, presents a tuft of hairs in the principal opening, being formed by the preputial ostium, parietal and visceral sheet. In the male bovine and buffalo, it is long with a reasonable opening allowing the passage of one or two fingers, with a cavity measuring between 25 to 40 cm in length by 3 cm in diameter. The protractor and retractor muscles are palpated in the superior inguinal region.

# Scrotal sac

The scrotal sac must be observed as to shape, texture, temperature and sensibility of the skin. Related aspects with the presence of depigmentation, photosensibilization,

Table 1 - Principal alterations of the prepuce and of the penis, detectable in the clinicoandrological examination

Eversion of the prepuce	Short penis or hypoplastic
Prolapse of the prepuce	Penis in a corkscrew shape
- Phymosis	<ul> <li>Ventral or lateral bypass of the penis</li> </ul>
➡Paraphymosis	Traumatize by rings of hairs or foreign body
Persistence of the preputial phrenule	Adherence of the penis to the prepuce
-Balanopostitis	Fracture, dilaceration with hematoma
-Acrobustitis-phymosis	Tumors in the prepuce or in the penis

ectoparasites, dermatitis, eczema, scars, lesions, hemorrhage, traumatisms, as well as neoplasias must be taken into consideration. In the bovine and buffalo, any alteration of the normality of the scrotal sac must be taken into consideration as to the quality of the semen due to the fact of the alteration in the thermo-regulation mechanism of the testicle, with increase temperature and consequently affecting the spermatogenesis. In the bovine, the scrotal sac normally presents certain rugosity, that develops according to the age of the bull, being an indicative of the proportion of interstitial tissue in relation with the parenchimatous tissue, or in other words the quantity of seminiferous tubes, (Blanchard et al., 1996). Thus, in bulls with excellent semen production presents a fine rugosity, while animals with a decrease in the quality of the semen presents an increase grade of rugosity.

# Testicles and epididymis

The testicles and epididymis are inspected and palpated together with the scrotal sac, that in the bovine and buffalo, presents a vertical presentation, in relation to the inguinal region. The inspection must be done with the animal free and with tranquillity, registering the presence of the testicles, shape, localization symmetry and size, that are confirmed by palpation that must be done with the animal restrained. The testicles must stick to the scrotum, localized vertically or horizontally. The palpation must be done with the animal correctly restrained, from behind, with both hands at the same time, take each structure individually between the thumb and the

other fingers, in a way to compare the morphology of them, pressing them gradually along the whole testicular surface (Lagerlof. 1934). Through the palpation of the testicles, besides to indicate the size, symmetry and consistence, permits to differentiate the existence of acute inflammatory processes, chronic or degenerative, beside the bypasses related with the alteration of the consistence. The size of the testicles must be approximately equal, however little differences are accepted in all species, while evident asymmetries must be taken into consideration, because it certainly means pathological conditions. Within all the related parameters with the size of the testicles, the testicular circumference is most important, because in bovine it is known to have a high correlation (r = 0.81) between the scrotal diameter and the sperm production (Coulter et al., 1976).

#### Size and consistence of the testicles

The testicles must be symmetrics. even though a slight asymmetry is not always consider an abnormality. The size of the testicles must be correlationed with the body size, fertility and sexual activity of the animal, whereas seasonal reproductive animals presents alteration in the size according to the season of the year. The shape of the testicles varies with different species from oval to round, while in bovines, it can be slightly long, having a cylindrical shape, principally in zebus. The testicular consistence have a fundamental meaning for the andrologic examination, because it reflects the production capacity and yet can indirectly foretell about the testicular content, parenchima's conditions and the intertesticular tissue or the existence of pathologic conditions. The testicle with a normal function presents resistant elastic consistence or tense-elastic, which confirm a normal activity of the spermatogenic process, with the seminiferous tubes being scarce of cells from the sperm cells lineage in division and with spermatozoids being produced normally. In the degenerative alterations of the spermatogenic process, that provokes alterations in the different cells of the spermatic lineage, affecting principally the spermatogenesis, the elastic consistence of the testicles substituted by a mild-elastic consistence or spongy, according with the state and duration of the process (Lagerlof, 1951 - 1963; Krause, 1966).

The consistence is measured by means of manual palpation, that can be place in the following score: 3 = resistant elastic or tense elastic consistence: 2 = elastic consistence; 1 = mild elastic or little elastic consistence; 0 = spongy or flaccid consistence. At the end of the palpation of the testicles, it is necessary to distinguish the presence of fluctuations - hydrocele or hematocele, abscesses, besides the abnormal consistence that characterize by being hard or firm (fibrosis), that means a severe stage of substitution of the normal testicular parenchyma by cells of the fibrous conjunctive tissue as well as mild or spongy (indicating a degenerative process) that denotes the beginning of a testicular degeneration process. The presence of granulation's tissue correspond to a increase of the interstitial tissue in the testicular parenchyma. product of a degenerative or advance inflammatory process (Lagerlof, 1951; 1963). In the palpation of the epididymis, firstly you must have in mind the topographic localization of the organ in relation to the correspondent testicle, observing the shape and size, position and inflammatory abnormalities or segmented aplasia, spermatic granuloma, that causes hardening of the organ. In ruminants, the epididymis head is found in the superior part of the testicle, having a plane triangular shape, covering dorsally the proximal part of the testicle, presenting a more firm consistence than that of the testicular parenchyma, beside a small gap that separates both structures. The body of the epididymis is found situated in the proximity's of the scrotal rafe, medially to each correspondent testicle, that can easily be

palpated raising up the testicle counter-lateral that is going to be palpated, which presents a thickness less than 0.5 cm, that is similar to the end of a chicken's feather. However, the part of the epididymis more easily palpated is the tail, that by its size and consistence it is possible to foretell the quantity of sperm cells stored and to correlation this finding with the concentration of the ejaculated. The clinical examination of this region terminates with the palpation of the functes or spermatic cords, that contains the deferent ducts, spermatic artery and vein, pampiniform plexus, lymphatic, cremaster nerves and muscle. The spermatic functes are palpable forcing both hands in a superior sense, above each testicle, observing the mobility, thickness, sensibility, edema, dilation of the vessels with stasis (varicocele), accumulation of adipose tissue, inflammatory processes or inguinal hernias.

 Table 2 - Principal alterations of the scrotal sac, testicle, epididymis and spermatic cord, detectable in the clinico-andrological examination

Rotation of the scrotal sac.	<ul> <li>Orchitis, periorchitis, epididymitis,</li> </ul>
🖛 Dermatitis, scars, traumatism,	funiculitis.
eczema, ectoparasites.	➡Hydrocele, hematocele
➡Scrotal hernia.	Testicle tumors.
►Varicocele.	-Epididymal dysfunction due biochemical
🖛 Anorchidia / criptorquidism.	alteration in the epididymis plasma, etc
🖛 Testicular hypoplasia - different types	-Segmented aplasia of the epididymis.
<ul> <li>Testicular degeneration.</li> </ul>	➡Spermatocele.
Adherence between the testicle	-Spermatic granuloma.
and the enveloping membranes.	

## Specific disturbances of epididymal function

Gustafsson (1966) described a dysfunction in the epididymis associated with reduced sperm motility, an increased frequency of sperm tail abnormalities, detached sperm heads or proximal cytoplasmic droplets. For Ramamohana Rao (1972) the epididymal dysfunction can also be associated with high glycerophosphorylcholine or with abnormal Na<sup>-</sup> or K<sup>-</sup> levels in the epididymal plasma, Gustafsson et al. (1972), or associated with a high levels of oestrogen, Cripps & Briggs, (1965) or with lower sperm production (Ramamohana Rao, 1972; Galloway, 1976). such problems have been described in Zebu bulls and buffalo at Brazil conditions (Vale Filho 1978 and Ohashi et al. 1988).

#### Spermatic cord

Throughout its structure the deferent duct, vessels and nerves correspondent to each testicle, beside the sheets of the vaginal membrane and the internal cremaster muscles. In this region is bund a vascular conjunct originating from the testicular veins and arteries, that form the pampiniform plexus, that is of great importance in the testicular thermo-regulation.

## Clinical examination of the internal genital organs

Examination of the internal genital organs is done by the rectal route, with the introduction of the whole hand and arm, being possible to palpate the ampoule of the deferent ducts, seminal

vesicle glands and prostate body, while the bulbo-urethral glands or Cowper's can be palpated indirectly in some animals or in pathologic cases.

# Vesicular glands or seminal vesicles

They are paired glands, being compact and lobulated in both species. In an adult bull, it measure between 10 to 12 cm in length to 4 to 5 cm in width and 2 to 3 cm in thickness, in an "S" shape, being more lobulated and smaller in size in buffalo. The excretory duct of the seminal vesicles empty in the ejaculatory ostium localize in the urethra. In cases of inflammation, due to the increase in size and sensibility of the affected gland, the possibility of palpation becomes more facilitated (Galloway, 1994).

# Prostate

Presents in two distinct parts depending on the specie, presenting a lobulated part, that form a cup, and the other externally that disseminate in a round shape and deeply along the urethral muscle, forming a disseminated part. In bovine and buffalo the prostate body is small and can be palpated.

# Ampoules of the deferent ducts

The ampoules of the deferent ducts presents itself in the bull with cylindrical structures in a pencil shape, of firm elastic consistence, measuring between 3 to 5 cm in length and 0.5 cm in diameter, while in buffalo the structures are similar to bovines, however, with a smaller size, being easily identified by rectal examination in both species.

# Internal inguinal rings

They are round structures, localize in a latero-superior position in relation to the pelvic floor, where the spermatic funicles pass in its pelvic localization. In bovines, these structures must be examined, facing a possibility of hernia's existence, and the viscera entrance in the formed cavity, that even though it is rare, it can happen.

# **Bulbo-urethral glands**

They are paired structures localized parallel to the rectum, in the ischiatic arch level, covered by bulbo-cavernous muscles. They are impossible to be reached by the clinical examination in bovine, however, possible in buffaloes. The secretion of these glands, together with the urethral glands, form a transparent liquid that is secreted initially when there is a sexual stimulation of the male, serving as an asepsis and lubrication for the urethra.

# Semen picture - spermiogram

The sperm cell defects can be hereditary or congenital as well as acquired due environmental factors. The sperm cell defects may be classified as head, middle piece and tail as well as protosplasmatic droplets and also foreign cells out of the normal epithelium of the testicles. These abnormalities can be divided in **primary** or **major** which are related to disorders in the seminiferous or germinal epithelium **secondary** or **minor** that occur after their passage



the determine ducts and balloo areana grands	
Segmented aplasia of the vesicular	Inflammatory process of the
glands	vesicular glands - spermatocystitis
Segmented aplasia of the ampoules	-Inflammation of the prostate -
of the deferent ducts	prostatitis, common in canine and equine
Masculine uterus	<ul> <li>Prostatic hyperplasia</li> </ul>
Segmented aplasia of the bulbo-urethral	Inflammation of the bulbo-urethral glands
glands	

**Table3**. Principal alterations of the internal genitalia, vesicular glands, prostate, ampoules of the deferent ducts and bulbo-urethral glands

through the whole genital system (Lagerlöf (1936-1963-1964), Haq (1948-1949), Blom 1964: Galloway, 1976; Leidl, Stolla & Schefels, 1996).

## Functional examination (sexual behavior)

## The sexual libido

The test of sexual libido is well studied in bovines, being done by direct placing of a male before a female in heat, and according to Chenoweth (1980), must have a duration between 5 to 10 and 10 to 20 minutes for Europeans and zebus breeds respectively. 0=without interest; 1=sexual interest demonstrated once; 2=sexual interest demonstrated more than once; 3=persistent interest in the search; 4=one mount or try without service; 5=more than one mount or try, without service;

6=mount and service. Reaction time, that correspond to the interval between the first contact with the female and the first mount try, with a reproducer can be classified according to the reaction in: 0 to 1 = C => poor / unsatisfactory; 2 to <math>4 = B => regular / moderate; 5 to <math>6 = A => very good / satisfactory.

# References

- Albihn, A. Maternal influence on the early embryonic development in the bovine. Sveriges Lantbruksuniversitet. PhD Thesis, Uppsala, 1991.
- Arthur, G. H., Noakes, D.E., Pearson, H. & Perkinson, T. J. Veterinary reproduction and obstetrics. 7th Ed., Saunders Company, London, 1996, 725 p.
- Bennetts, H. W., Underwood, E. J. & Shier, F. L. A specific breeding problem of sheep on subterranean clover pastures in Western Australia. Aust. Vet. J. 22:2, 1946.
- Blockey, M. A. B. Sexual behavior of bulls at pasture: a review. Theriogenology 6(4):38-392, 1976a.
- Blockey, M. A. B. Serving capacity a measure of the serving efficiency of bulls during pasture mating. Theriogenology 6(4):393-401, 1976b.
- Blanchard, T. L., Varner, D. D., Bretzlaff, K. N., Morris, D. L., & Elmore, R. G. Mate reproductive disorders. In: Large animal internal medicine. Bradford Smith, Ed. Mosby. St. Louis, 1996, p. 1566-1583.
- Chenoweth, P. J. Libido and mating ability in bulls. In: Morrow, D. A. Current therapy in theriogenology. Phyladelphia, W. B. Saunders, 1980, p. 342.
- Coulter, G: H., Rounsaville, T. R. & Foote, R. H. Herdability of testicular size and consistency in Holstein bulls. J. Anim. Sci., 43:9, 1976.

- Cupps. P. T.& Briggs, J. R. Changes in the epididymis associated with morphological changes in the spermatozoa . J. Dairy Sci 48:1241-1965.
- Drost, M. & Thomas, P. G. A. Diseases of the reproductive system. Female reproductive disorders. In: Large animal internal medicine. Bradford Smith, Ed. Mosby, St. Louis. 1996.
- Galloway, D. B. A study of bulls with the clinical signs of seminal vesiculitis. Acta Vet. Scand. 5 suppl. 2, 1964.
- Galloway, D. B. Factors affecting fertility in bulls. Proc. 2nd. National Symp. Anim. Reprod., Belo Horizonte, 1976, p.102-122
- Götze, R. Besamung und Unfruchtbarkeit der Haussäugetiere. Verlag M. & M. Schaper, Hannover, 1949, 613 p.
- Grunert, E. & Berthtold, M. Fertilitaetstoerungen beim weiblichen Rind. Verlag Paul Parey, Berlin, 1982, 522 p.
- Grunert, E. & Birgel, E. H. Obstetrícia veterinária. Sulina, Porto Alegre, 1982
- Grunert, E. & Gregory, R. M. Diagnóstico e terapêutica da infertilidade na vaca. Sulina, Porto Alegre, 1984, 163 p.
- Gustafsson, B. Luminal content of the bovine epididymis under conditions of reduced spermatogenesis, luminal blockage and certain sperm abnormalities. Acta Vet. Scand., Supp. 17, Stockholm, 80p. 1966.
- Gustavsson, I. & Rockborn, G. Chromosome abnormality in three cases of lymphatic leukemia in cattle. Nature, 203:390, 1964.
- Haq, I. Studies on tissues cells (other than spermatozoa) of bull semen. 1st. Int. Cong. Phys. Path. Anim. Reprod. & A. I., Milano, 1st. Sec., p. 1-26, 1948.
- Haq, I. Cause of sterility in bulls in southern England. Br. Vet. J. 105:3-6, 1949
- Johnson, H: D: The lacting cow in the various ecosystems: Environmental effects on its productivity. Feeding dairy cows in the tropics. FAO Animal Production and Health Paper 86. Rome, 1991, p. 9-21.
- Joosten, I., Stelwagen, J. & Dijkuizen, A.A. Economic and reproductive consequences of retained placenta in dairy cattle. Vet. Rec. 123(2):53, 1988.
- Krause. D. Untersuchungen am Bullensperma unter Berücksichtigung der fertilitätsdiagnostichen Bedeutung der Befunde. Tierärztlichen Hochshule Hannover, Habilitationsschrift, 1966, 165 p.
- Kuntze, A. & Aurich, J. Der Endometritis-Pyometra-Komplex bei Tieren. Gustav Fischer, Stuttgart, 1995.
- Lagerlöf N. Morphologische Untersuchungen ueber Veraenderungen in Spermbild und in den Hoden bei Bullen mit verminderter oder aufgehobener Fertilitaet. Acta Pathologica Microbiologica Scandinavica, Supplement, XIX, 1936, 254 p.
- Lagerlöf. N. Investigation of sterile bulls. FAO-Swedish Int. Post-Grad. Course on Animal Reproduction. Royal Vet. College, Stockholm, 1963a, 9 p.
- Lagerlöf, N. Testicular degeneration. FAO-Swedish Int. Post-Grad. Advanced Training Center on Anim. Reprod. Royal Vet. College, Stockholm, 1963b, 13 p.
- Lagerlöf, N. Semen examination as an aid to sexual health control in domestic animal breeding. Int. J. Fert. 9(2):377, 1964.
- Leidl, W., Stolla, R. & Schefels, W. Sperm morphology. Minitub of America, CD-Rom, 1996.

- Long, S. Abnormal development of the conceptus and its consequences. In: Arthur, G.H.; Noakes, D.E.; Pearson, H.; Parkinson, T.J. Veterinary Reproduction & Obstetrics. 7<sup>th</sup> ed., W.B. Saunders. London, 1996.
- Lopez-Diaz, M. C. & Bosu, W.T.K. A review and an update of cystic ovarian degeneration in ruminants. Theriogenology 37:1163-1184, 1992.
- Lotthammer, K. -H. Influence of nutrition on reproductive performance of the milk/gestating cow in the tropics. Feeding dairy cows in the tropics. FAO Animal Production and Health Paper 86, Rome, 1991, p. 36-47.
- McEntee, K. Reproductive pathology. Academic Press, San Diego, 1990.
- Megale, F. Aspectos clínicos das malformações congênitas da genitália bovina. Tópicos avançados em reprodução animal. Sociedade Brasileira de Genética, Ribeirão Preto, 1980, p. 43-68.
- Merkt, H. & Krause, D.Die Andrologische Untersuchung. Merkblatt 13a, 13b, 14. Klinik für Andrologie und Besamung der Haustiere, Tierärztlichen Hochshule Hannover, Hannover, 1977, 6p.
- Mamont, H: W: , Seguin, B. E., Singh, G. & Stasiukyans, E. Does intrauterine site of insemination in cattle really matter? Theriogenology 32:19, 1989.
- Morrow, D. A. Current therapy in theriogenology (Diagnosis, treatment and prevention of reproductive diseases in small and large animals) 2nd Ed. 1986
- Noakes, D. E. Infectious forms of infertility in cattle. In: Arthur, G: H.; Noakes, D. E.; Pearson, H.; Parkinson, T. J. Veterinary Reproduction & Obstetrics. W. B. Saunders, London, 1996.
- Nordlund, S. A new type of genital malformation in Swedish friesian cattle. Proc. 3rd Int. Cong. Anim. Reprod. and A. I., 2:80, 1956.
- Ohashi, O. M., J. S. Sousa & W. G. Vale. Aspecto reprodutivo no macho bubalino. In: W. G. Vale (Editor). bubalinos: fisiologia e patologia da reprodução. Editora Cargill, Campinas, 1988, p. 69-86.
- Paisley, L. C., Mickelsen, W. D. & Anderson, P. B. Mechanism and therapy for retained foetal membranes and uterine infections of cows: a review. Theriogenology 25(3):353, 1986.
- Pinheiro, L. E. L. Estágio atual da genética médica veterinária no Brasil. In: Tópicos avançados em reprodução Animal. 1. Simpósio Nacioanl, Jaboticabal, 1980, p. 7-14.
- Revista Brasileira de Reprodução Animal, Padrões para o comércio de sêmen e embriões no Mercosul. Rev. Bras. Reprod. Anim. 20(1):2, 1996.
- Ribeiro, H. F. L. & Vale, W. G. Patologia da reprodução an búfala, Bubalus bubalis Lin. Bubalinos: fisiologia e patologia da reprodução. Fundação Cargill, Campinas, 1988, p 39.
- Roberts, S. J. Veterinary obstetrics and genital diseases.(Theriogenology). 3 rd. Ed. 1986
- Ramamohana Rao, A. Changes in the morphology of sperm during their passage through the genital tract in bulls with normal and impaired spermatogenesis. Royal Veterinary College, Stockholm, PhD Thesis, 1971.
- Senger, P. L., Becker, W: C: & Davidge, S. T. Influence of cornual insemination on conception in dairy cattle. J. Anim. Sci. 66:3010, 1988.
- Shafie, M. M. Physiological responses and adaptation of water buffalo. In: M. K Youssef, Editor. Stress Physiology In Livestock, vol. 2: Ungulates, C. R. C., Florida, 1985.
- Shafie, M. M. Environmental effects on water buffalo production. World. Animal Review, 77:21-25, 1993-1994.
- Spriggs, D. N. White heifer disease. Vet. Rec. 58:415-418, 1946.

- Vale, W. G., Ohashi, O. M., Ribeiro, H. F. R. & Sousa, J. S. Causes and incidence of infertility and subfertility in zebu crossed cows in amazon region of Brazil. Vet. Med. Res. 2:133, 1984.
- Vale, W. G., Ohashi, O. M., Sousa, J. S. & Ribeiro, H. F. L. Clinical reproductive problems in buffaloes in Latin America. In: II. World Buffalo Congress. New Delhi. Proc. Vol. II, Part I, 1988, p.206-217.
- Vale, W. G., Ohashi, O. M., Ribeiro, H. F. L. & Sousa, J. S. Semen freezing and artificial insemination in the water buffalo in the amazon valley. Buffalo Journal, Bangkok 7(2):137-144, 1991.
- Vale, W. G. Reproductive management of water buffalo under amazon conditions. Buffalo Journal, Bangkok, 10(2):85-90, 1994.
- Vale, W. G. & Ohashi, O. M. Problems of reproduction in buffaloes. Buffalo Journal, Bangkok, Suppl. N. 2, p. 103, 1994
- Vale, W. G. Reproductive management of water buffalo under amazon conditions. Buffalo Journal 10(2):85, 1994.
- Vale Filho, V. R.; Pinto, P. A.; Fonseca, J. & Veiga, L. C. S.. Patologia do sêmen. diagnóstico andrológico e classificação do Bos taurus e Bos indicus quanto à fertilidade para uso como reprodutores em condições de Brasil - De um estudo em 1088 touros. Monografia, 54 p., 1978
- Woolums, A. R. & Peter, A. T. Cystic ovarian conditions in cattle. Part I. Folliculogenesis and ovulation. The Comp. Cont.Edu. 16(7):935-942, 1994.