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BUFFALO REPRODUCTION AND BREEDING IN BRAZIL

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Abstract

This review focuses on the main aspects of buffalo reproduction and breeding in Brazil. Characteristics are described in groups, breeds and types of buffalo with special attention to the introduction of this species about a century ago and the crossbreed phenomena between river (2n=50) and swamp (2n=48) types, giving origin to a 2n=49 hybrid. Breeding seasons are not observed in the Amazon basin conditions, and buffaloes can be bred and calved year round, with this characteristic being controlled by the availability of nutrition, however in other regions of the country there is a marked seasonal influence. Puberty in the female can be reached at the age of 16-20 months, and 20-24 months for river and swamp types, respectively. The estrous cycle has a length 22-24 days. The gestation period is between 300-310 days for river types and 310-330 days for swamp types and calving intervals varying between 12-14 months, can be attained. Artificial insemination has been accomplished using deep-frozen semen from national and imported bulls and fertility rates up to 70 percent in semi-extensive reproductive management and 85 percent in well managed herds with nutritional supplementation can be achieved. For beef production, buffalo are superior to those performances obtained for bovine and it is possible to obtain a buffalo male weighing more than 400 kg of live weight at two years of age raised on native floodplain pasture. For milk production there is great future in selecting females that can give more than 2,000 kg of milk in 270 days of lactation. Draught animal production is another economic performance that should be emphasized as important mainly among small-holder farms. The effective utilization of genetic resources and introduction of "new" germoplasma in the semen form is a new strategy for widespread use in this species. Thus, buffaloes are a good indicative for new sustainability strategies in the optimization of the locally-available resources and reduced deforestation in livestock production in the Amazon region.

Key words: reproduction, breeding, buffaloes

Introduction

Brazil is a tropical country, with an area of 8,511,965 square kilometers, bordering every other country of South America with the exception of Chile and Ecuador, with a plateau varying from 40% lower than 200 meters, 57% between 200 to 900 meters and only 0,5% upon 900 to 1.200 meters. Almost 93% of the country is within the tropical zone, with the bulk of the territory lying between the Equator which crosses the northern mouth of Amazon and the Tropic of Capricorn crossing downwards through the city of São Paulo.

The country is divided into five regions: the North or Amazon which covers an area of 4.9 million square kilometers which comprises 57% of Brazil's territorial land, the Northeast, the Southeast, the South and the Midwest.

Climate

The climate is most tropical with some sub-tropical zones in the south, usually divided into winter and summer, corresponding roughly to a dry and wet season which is more prominent in south-central and southern areas where seasonal temperature changes are more pronounced. The effects of latitude, however, are modified by a number of factors, such as altitude, prevailing winds, rainfall and distance from the ocean. and it is largely determined during the year by successive air masses of different origins (air masses composed of a large volume of air of uniform physical properties, especially with regards to humidity and temperature). The air masses and pressure centers on their part are determined by the path of the sun, above all by its zenith at midday. In March and September the sun's highest point is over the Equator and in December it is directly above the Tropic of Capricorn. In the Humid Tropical Amazon region, the low altitude above sea level and proximity to the Equator cause abundant rainfall between 1,000 and 3,500 mm per year and only the marginal zones further from the Equator have below 2,000 mm per year and constantly high temperatures which hardly vary more than 2-3^{o} C during the year from the mean value of about 24,5^{o} C, providing in this way a typical feature of a tropical region. Moreover, rainfall also varies with regard to different areas, with frequent thunderstorms due to the heat, and heavy showers every day during the early afternoon or even in the early morning or other hours of the day. Furthermore, another very characteristic feature of the entire region is the extremely high humidity of the air, which is always above 80% and in the interior of the forest, is often only a little below 100 per cent. In south-central and southern areas, seasonal temperature changes are more pronounced and freezing temperatures can be found in some areas between April and July, with snow falls in high and mountainous areas.

Vegetation

The natural vegetation of Brazil has remained to a very large extent in its original state despite the deforestation caused by the occupation of new areas. It is largely determined by the climate and also by the soil. In 1995 arable land usage was allowed in more than 3.5 million km^2. This figure includes all cultivated land, bush and tree plantations as well as the usually intensively used pastures, (Figure 1). Thus almost the entire Amazon Basin is dominated by the tropical-equatorial forest which can be divided into three types, depending on their position with regard to the rivers. The first one is the true forest or "terra firme" which has been destroyed and cleared for timber and fodder production and normally inappropriate to use due to large parts of its organic material lost. With that many valuable nutrients are quickly washed away by the tropical rainfall, the thin humus layer is destroyed and the infertile soils falls prey to further erosion. Also the forest deforestation interferes with the water cycle, since in this region a large part of the precipitation is formed by water that has evaporated from the leaves of trees in the tropical forest. So when these trees are destroyed and lacking in great numbers, the water necessary for rainfall is restricted. However, in the floodplains there are more than

25million hectares of native pasture. An important source of fodder consists of floating *canarana*, formed by a group of robust grasses that grow along the banks of rivers, streams, ponds and lakes. These vast areas of seasonally-inundated swamp land of the Amazon river and its tributaries, constitute low-grade pasture barely able to support any other grazing animal and was recognized by Cockrill as "buffalo paradise" and their utilization by the grazing buffaloes offers the only possibility for a profitable returns from the land (Cockrill, 1986).

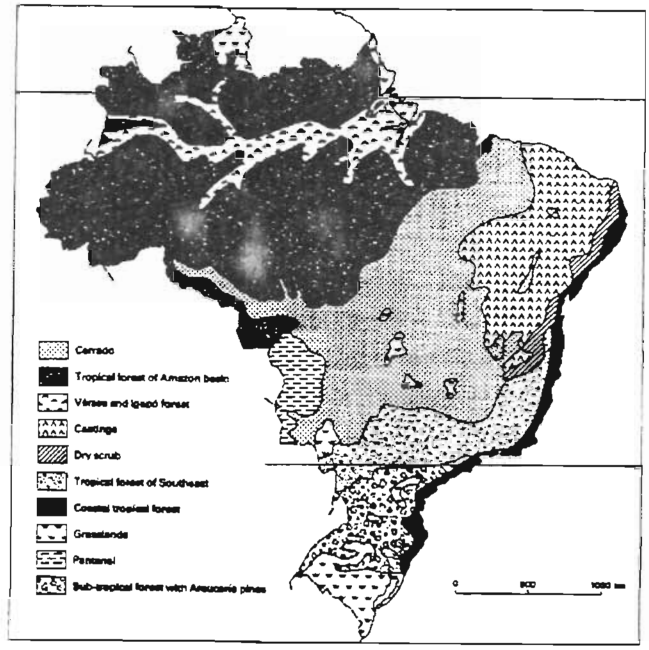


Figure 1 - Presentation of the main vegetation's areas of Brazil.

In the Northeast region is drought and half are localized in the semi-arid zone or "Sertão" with irregular periods of rain Most of the vegetation is formed by "Caatinga" or "Cerrado". These areas are not acquired for large animal production although in the riverbanks and in some individual mountain ranges it is possible to breed all types of farm animals. The southeast region includes an area where, traditionally, buffaloes have been raised together with cattle in extensive and semi-extensive ranching systems This area has vast quantities of cultivated pasture, mainly of *Pannicum maximum*, *Brachiaria bizanthia*, *Melinis multiflora* and other species. Buffaloes in the southern region have been raised in areas where the other domestic species like cattle and sheep cannot be raised economically, especially in swampy and poor soil areas. The midwest region is comparable to the Amazon region, with main emphasis to the swamp and cerrado areas of Pantanal, Figure 1.

Buffalo origin, groups, breeds and types

The buffalo came to Brazil at the end of last century from southeast Asia and Italy. In 1895, a farmer by the name of Vicente Chermont de Miranda, officially imported a herd from Italy to Marajó island. Further importation were made from Italy and India. It is estimated that the buffalo population in Brazil is approximately 3 million head with half of this total in the Amazon basin, more than 1.5 million and showing an annual increase of 13 percent. The high fertility level ensures that most of the mature females are pregnant most of the time, showing an average calving interval of 13 months. Buffaloes have been traditionally grouped, together with cattle and other oxen, in the bovine, sub-family of the family Bovidae. There are two main groups of domestic buffalo, the Genus *Bubalus*, species *Bubalus bubalis*, including the "river" or "dairy" type with (2n=50) and "swamp" type or Carabao, with (2n=48) chromosomes Table 1 and Figure 2. These findings suggest that the karyotype evolution is based mainly on centric fusion's, arising from Robertsonian translocations involving some chromosomes and both types producing offspring. However, in a study involving 152 buffaloes crossed between river and swamp types (Guimarães, 1990), a tendency was

Table 1 - Chromosome features in buffalo raised in the Amazon basin.

Type	Chromosome number	Chromosome feature
•Indian. river milk or Mediterranean type	2n=50	5 metacentric, 19 acrocentric and the sexual acrocentric
•Swamp or carabao type	2n=48	5 metacentric or submetacentric, 18 acrocentric and the sexual acrocentric
•Crossed indian x swamp	2n=49	5 metacentric, 18 acrocentric with a fusion between the chromosomes 4/9 and the sexual acrocentric

Source: Laboratório de Reprodução Animal - UFPA (unpublished data)

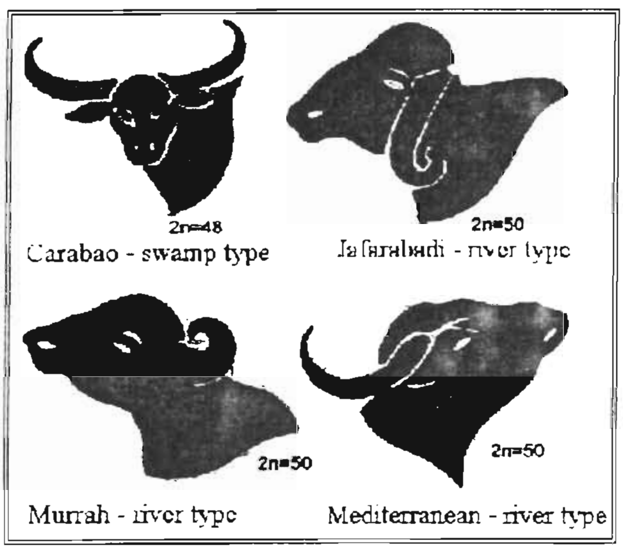


Figure 2 - Breed of buffaloes raised in Brazil.

observed towards stabilization in incompatibility due to aneuploid structures and assynapses. In the male crossed animals, the population of seminiferous tubules which completed the normal spermatogenesis was higher in (2n=50) than in (2n=49) animals. Furthermore, the presence of multi and univalent structures in meiosis of (2n=49) animals chromosome, gave origin to aneuploid gametes which can of course affect the fertility of the animals. However, the author concluded that more studies are necessary for a clear conclusion and determination about how much the level of fertility is affected.

Reproduction in the female

Morphology of the female reproductive tract

The female reproductive tact in buffaloes of both swamp and river types are similar to those in the *Bos taurus taurus* and *Bos taurus indicus*, Table 2.

Puberty

This can be defined as the physiological stage of an animal where sexual functioning ability is attained. It is not associated with the complete anatomical development of the body but expressed whenever physiological functioning of some endocrine glands are normally complete, together with the minimum attainment of the structural development of the female reproductive tract is. The female *Bos taurus taurus*, for example a Holstein heifer reaches puberty at 9-19 months of age. In zebu *Bos taurus indicus*, as well *as* in buffalo, it can reach around 20 months. However in favorable environment and with good nutritional management, both buffalo and zebu heifers can reach puberty at 16 months of age with body weight greater than 300 kg. Nevertheless if feeding standards are not optimum, puberty is not reached earlier than three years of age. Based on assessments of Progesterone concentrations it was found that Murrah x Mediterranean heifers raised on extensive condition of artificial pasture of koronovia grass (*Brachiaria humidicola*) reach sexual maturity between 2.1 and for Carabao heifers 3.3 years (Vale et al. 1990 Vale. 1994). However, at optimal nutritional levels and management, puberty can be reached at 16 months of age. However Carabao heifers are less precocious and only attain puberty after 24 months of age.

Table 2 - Comparative patterns of the reproductive tract of the female buffalo and bovine

Description	Buffaloes	Bovine
Ovary length (cm)	2.2-3.0	2.8.3.8
Ovary weight (g)	3.0-4.0	5.0-9.4
Number of primordial follicles in each ovary at birth	12.000-20.000	60.000-100.000
Diameter of the mature follicle (cm)	1.4-1.7	1.9-2.2
Corpus luteum	Brown-red, not bulging to the surface but growing into the medullar	Yellow, bulging to the ovary surface forming a crown
Diameter of mature corpus luteum (cm)	1.3-1.6	1.7-3.0
Weight of mature corpus luteum (g)	0.7-1.5	1.2-2.5
Oviducts	19-23 (cm) length	25-26 (cm) length
Uterine horns - length (cm)	30-38: also more rigid and tonnus more evident than in bovine	35-45
Uterine body diameter (cm)	2.1-2.8	2.5-5.0
Cervix	5.3 - 7.1 (cm), also its narrower and the lumen more tortuous in the cow	6.5.-12.7 (cm)
Vulva	Presence of hair in the external lips which can accumulate yellowish urate salts	

Adapted from Luktuke & Rao, (1962); Mobarak, (1969); Dobson & Kamonpatana (1986), Danell (1987) and Vale et al., (1988).

The non availability of food seems to be the most important constraint for anoestrus in the female. In Amazon basin they can produce a calf per year, the female is productive for 25 or more years and the herd is increasing 13 per cent yearly (Vale, 1996). Also buffaloes are very well managed in backyards in intensive breeding or feedlot-like conditions, where they can be used for milking. Based on analysis of sequential milk, progesterone assessment during the post-partum period, it was found that first ovulation occurred 30.2 ±14 days in buffalo cows under improved management; under traditional management conditions this was delayed until 102.4±42.7 days of the postpartum period, making it impossible in many cows to achieve yearly calving intervals (Vale, 1988).

Reproductive endocrinology and oestrus cycle

The reproductive endocrinology of buffaloes shows some difference when compared with bovine, as can be observed in the Table 3.

Reproductive patterns

Although many authors have considered buffalo as essentially a seasonal breeder, it has been proven that this species is a polyoestrus animal, mainly in the humid tropical areas of the world and especially in the Amazon basin (Vale et al., 1990). However, in temperate areas some reproductive functions are characterized by marked seasonal variations (Zicarelli, 1994).

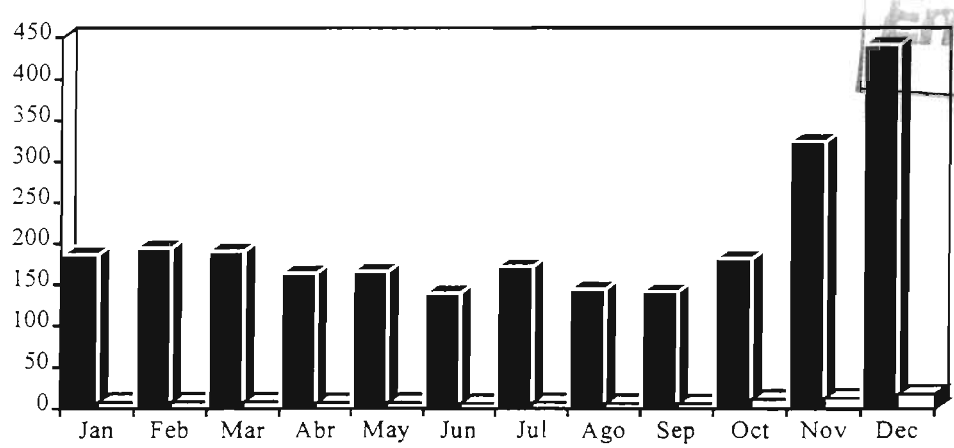
Table 3 - Comparative patterns of hormonal picture in the female buffalo and bovine

Hormone	Buffalo	Bovine
Progesterone - peak in the luteal phase	Blood plasma & whipped milk: 1.5-5.0 ng/ml;	Blood plasma & whipped milk: 4.0-7.0 ng/ml;
17β-Estradiol	25-32 pg/ml River type 8-10 pg/ml swamp type	25-30 pg/ml
FSH	12 ng/ml around the cycle; maximum secretion 60 ng/ml day 0 of estrus	Irregular fluctuation waves through the cycle varying between 10-400 ng/ml, with a peak maximum of 78 - 600 ng/ml
LH	072 -2,0 ng/ml around the cycle, 20-40 ng/ml in the peak	1,7 - 7 ng/ml around the cycle, with a peak maximum of 15 - 65 ng/ml
15keto-dehidro PGFM	250 - 900 pg/ml 2-3 days before the estrus	150 - 570 pg/ml 2 - 3 days before the estrys

Adapted from Dobson & Kamonpatana (1986), Danell (1987) and Vale et al., (1988).

The absence of seasonal effect and the possibility to browse on excellent pasture found in the floodplain around the year, allow buffaloes to have a short calving interval, thus increasing reproductive efficiency, Figure 3. In these regions, the rate of increase is thought to be at least 13 percent per annum which is greater than that of any other domestic animal, anywhere in the world.

Figure 3. Annual distribution of buffalo calving in the Middle Amazon Region, 1993 (n=2.539)



Calving	**186**	**194**	**190**	**163**	**165**	**139**	**171**	**144**	**141**	**181**	**324**	**441**
(%)	**7,3**	**7,6**	**7,5**	**6,4**	**6,5**	**5,5**	**6,7**	**5,7**	**5,6**	**11,1**	**12,8**	**17,3**

Source: Vale et al. (1996)

The buffalo female has a gestation period of 300-310 days and can on the average give birth every 12-15 months in extensive management. However controlled husbandry practice is necessary, since the introduction and knowledge of good management is fundamental in obtaining higher fertility rates mainly when artificial insemination is used (Vale et al., 1991). In contrast, in a herd for where reasonable pastures were available all year round and supplements were provided, calving occurred throughout the year but was more frequent in November and December, with a regular distribution in the other months of the year Figure 3.

Table 4 - Number and frequency of age at the first service in Murrah x Mediterranean heifers

Age in days	Number of heifers	Frequency (%)
< 700	5	5.49
701 to 730	18	19.78
731 to 760	32	35.16
761 to 790	20	21.97
791 to 820	16	17.58
TOTAL	91	100.00

Source: Laboratório de Reprodução Animal - UFPA (unpublished data)

Table 5 - Number and frequency of body weight at time of first service in Murrah x Mediterranean heifers

Weight (Kg)	Number of heifers	Frequency (%)
300 to 329	3	6.66
330 to 359	6	13.33
360 to 389	11	24.44
390 to 419	19	42.22
420 to 450	6	13.33
TOTAL	45	100.00

Source: Laboratório de Reprodução Animal - UFPA (unpublished data)

Overall averages for some reproductive characteristics in river and carabao females are summarized in Table 6. The duration of the estrous cycle is 22-24 days for cows and 19-20 days for heifers and the length of the oestrus symptoms last for12-36 hours (Vale, 1988). Female buffaloes demonstrate peculiar behavioral changes during the oestrus cycle and the principal symptoms are bellowing, mucus discharge, vulvar edema and hyperemia, raising of the tail and frequent micturition in short spurts. All of these symptoms should be associated and taken into account together, Table 6.

Table 6 - Some reproductive characteristics in river and carabao females submitted a good nutritional management

Type	Sexual maturity. (days)	Oestrus length (hours)	Service. Period (days)	Gestation (days)	Calving Interval. (months)
River	16-20	22-24	30	300 - 310	12-13
Swamp	20-24	22-24	30	310 - 320	13-14

Source: Vale et al. (1994)

Heat detection should be carried out by a teaser buffalo bull fitted with a chinball marking device associated with close observation of female behavior and symptoms as stated in Table 7. The female in heat should be separated from the herd and insemination should be performed patiently and in the cooler hours of the day. It should be remembered that low reproductive efficiency as a result of artificial insemination is perhaps the outcome of bad management and poor nutrition, Table 8.

Inactive or non-functional ovaries is one of the most important causes of anoestrus in female buffaloes. It can be caused by nutritional disorders, relating to energy, protein and minerals or thermal stress which cause a blockage of hypothalamic-pituitary gonadal axis, leading to a lack of the circulatory hormones, FSH, LH and estrogen, which contributes to ovarian inactivity and thus anoestrus (Vale et al., 1988; Vale, 1988).

Table 7 - Percentage of oestrus symptoms observed during 88 heats of 70 water buffalo cows submitted to artificial insemination (A.I.).

Symptoms	n. of cases	%
1. Bellowing	51	57.9
2. Mucus discharge	67	76.1
3. Vulvar edema	59	67.0
4. Raising of the tail	84	95.4
5. Vulvar hyperemia	58	65.9
6. Mounting of other females	15	17.0
7. Mounted by other females	29	32.9
8. Frequent micturition in short spurts	59	67.0
9. Mounted by the teaser bull	88	100.0

Source: Vale et al. (1991)

Table 8 - Number of buffalo cows, number of first to third artificial insemination, total of semen doses used, number of doses per pregnant cow, number and percentage of calve born, in a program using imported semen - 1991-1992.

		Number of A. I.			**performed**	**Calving**		**rates**
Group	Cows	1st	2nd	3rd	Total	cow	n	%
I	18	18	10	02	30	1.7	17	94.4
II	24	24	12	-	36	3.0	12	50.0
III	21	21	8	7	36	3.0	12	57.0
TOTAL	63	63	30	09	102	2.58*	41	67.1*

*Average

Source: Laboratório de Reprodução Animal - UFPA

Reproduction in the male

Morphology of the male reproductive tract

The reproductive organs of the male buffalo is similar to those in bovine. The scrotal sac in the River breeds are large and pendulous than in Swamp male which is shorter, even when relaxed. The testicles in both River and Swamp are smaller than those of European and Zebus. The male sheath is much closely bound to the belly than in case of *Bos taurus indicus* and quite similar as in *Bos taurus taurus*. the penis is shorter and the way it is protruded is similar in the ruminants. The internal genitalia, prostate and seminal vesicles are smaller than in the bovine, with the seminal vesicles showing more small lobules.

Training males for semen collection

The male buffalo is perhaps the easiest domestic specie to be trained to serve the artificial vagina and generally it will ejaculate into the artificial vagina at the first attempt. Young males at puberty start to mount other males and flehmen response is observed when the bull sniffs the other herdmates male genitalia and urine, extending its chin and inhales with slight open mouth. The sexual behavior at artificial service is weak when compared to European breeds and the thrust given by bulls was not strong as was evident for European cattle. Buffalo can be conditioned to jump in a male or in a female, although the reaction time and time taken mounts as well. Temperament is not correlated with other seminal attributes like volume, concentration, mass activity and vigor (Vale, 1994).

Semen characteristic

Buffalo semen has problems associated with seasonal factors and the key enabling factor is the successful long-term storage of frozen buffalo spermatozoa in a good environmental temperature. In the case of tropical temperature as in Northern Brazil (Amazon region) is to impose a good management to take away the heat stress (Vale et al., 1991; Vale, 1994). For best results semen must be collected by artificial vagina at 44-45 ° C. Each collection consists of two ejaculations - first and second, collected within an interval of minimum 30 minutes. False mounts are recommended to increase the quality of the ejaculation. Immediately after the collection the ejaculate must be assessed for the followed parameters and then put in a water bath at 37 ° C (Vale, 1994).

Color or density

Depend upon variations in sperm cell concentration, thus certain degrees of color or density reflect certain concentration of sperm cells in the ejaculate. Generally the normal color of buffalo semen is variable from white, milky to white or creamy with light tinge of blue (Vale, 1994).

Volume

Variations in volume can occur in case of young and old bulls In our experience young bulls at puberty start with a semen volume around 1,0 ml which increase to >3ml after they reach sexual maturity. Attention must be paid in case of testicular degeneration caused by heat stress, when not only the volume but the other semen attributes tends to be altered. Volume of buffalo ejaculate is measured immediately following semen collection through the graduated collection tubes and it is less than in the bovine. The volume of semen obtained through the artificial vagina is variable according to the breed and age of the male. Young males give low volumes 1-3 ml per collection, moreover old bulls can ejaculate 6 ml or more (Vale, 1994).

Table 9 - Characteristics of a normal buffalo ejaculate collected through an artificial vagina, according to (Vale, 1994).

Semen pattern	
-Color	white, milk white, with light blue tinge
-Volume	3 ml (2 to 8)
-Wave or swirl motion	>3
-Motility (%)	>70
-Vigor (individual motility)	>3
-Concentration	6×10^5 to 12×10^5
-Live sperm (%)	>70
-Abnormal sperm (%)	<70
-pH	6.7 to 7.5

Concentration

Normal buffalo sperm concentration shows a wide range of variation (600.000 to 1,200,000 cells per mm^3 - 0,6 x 10^6 to 1,2 x 10^6 mm^3) and this parameter is highly sensitive to seasonal and nutritional disarrangement (Vale, 1994). Concentration is the sperm density commonly estimated per mm^3 of the semen volume. There are many different methods to count sperm concentration, however, the two most practical ones used are the haemocytomer and photo-electric colorimeter methods. An accurate sperm count can be obtained with haemocytometer as follows: Draw the semen into a glass Sahli pipette of 20 µl or an Eppendorf automatic pipette. This volume is diluted in a 4 ml of Hancock formol tamponed saline 1% or sodium citrate tamponed solution 1 %, giving a dilution of 1:200; a Neubauer chamber is used. and is filled as for blood cell counting. All the sperm cells in the five squares - four corner squares and in the entire ruled center square are counted. To calculate the total number of sperm multiply by 10,000 (Vale,1994).

Wave motion

Ejaculate wave or swirl motion is determined by observation of a rather thick drop of semen put on a clean and dry glass slide maintained at 37° C under low power magnification (x 10) and reduced light. The entire drop is observed for the presence of waves and eddies which is caused by the continuos movement of dark streaks in the field. Swirls appear and disappear in the extremities very rapidly. On the basis of the activity of swirls or mass activity semen is graded grossly into a score of (0 to 5), Table 10. A normal or good quality buffalo ejaculate shows frequently (>3), that means more than 60% of mass activity (Vale, 1994).

Motility

Sperm motility is judged as a gross motility of the sperm in a rapid way of evaluation and is commonly used. A small drop of semen is placed on a clean and dry glass slide maintained at 37° C and a cover slip is put on it and then assembled in the thermos-regulated stage of the microscope and examined at magnification(40 or 100 X), Table 11.

Table 10 - Descriptive and numerical scales for evaluation of microscopic waves pattern of semen from buffalo, according to (Vale, 1994).

Description scale	Numerical scale	Appearance pattern
-Very poor	0	-Waves not present; sperm cells immobile
-Poor	1	-Waves not present; motile sperm cells
-Fair	2	-Barely distinguishable waves in motion
-Good	3	-Waves apparent; moderate motion
-Very good	4	-Dark, distinct waves in rapid motion
-Excellent	5	-Very dark, many waves in all directions

Table 11 - Descriptive and numerical scales for evaluation of microscopic motility of sperm cells from buffalo, according to (Vale, 1994).

Motile cells - %	Descriptive value	Numerical value
80 - 100	Very good	5
60 - 80	Good	4
40 - 60	Fair	3
20 - 40	Poor	2
0 - 20	Very poor	1

Initial motility varies with the frequency and season of collection. Usually, when the routine semen collection is done twice a week, first and second ejaculate, generally both ejaculates show good motility (>3), with more than 60% of the sperm cells with normal progressive rectilinear motility.

Vigor

Vigor or individual motility is scanned for the field with the highest number of motile sperm cells. One sperm must be followed until it disappears from the area. This operation should be repeated many times. A good ejaculate must have (>3), which means more than 60% of individual motility.

Live and dead

Through the determination of live and dead sperm it is possible to predict the quality of the ejaculate. Semen with more than 30% initial dead spermatozoa may not be good for freezing and semen samples with less than 50% of live spermatozoa may be of questionable fertility even throughout natural breeding. Live percentage of sperm cells may increase in the second ejaculate and this is collected in regular intervals twice a week. Differential staining techniques have been utilized for counting live and dead spermatozoa in semen smears. Slides may be prepared for immediate use. The smears from each sample should be prepared and stained with Eosine 1 % stain. 100 sperm cells from each smear should be counted for live and dead cells.

Sperm morphology

The percentage of abnormal sperm in the buffalo ejaculate is an important point to be observed when the objective is to freeze the semen. Morphological study of spermatozoa is done through preparation of smears from freshly collected semen. The smears should then be stained through the use of suitable staining techniques. The carbo-fucsine-eosin stain method according to WILLIAMS, has been used successfully. The various types of structural abnormalities associated with head, middle piece, and tail are recorded. Those abnormalities may either be hereditary or be of developmental and of environmental nature. They can be classified as in the bovine according to their source and nature. Stained smears are examined under oil immersion (X1.000) and 200 sperms cells are counted. A second method is to examine a wet preparation of sperm fixed in formol tamponed saline solution mounted with a cover slip and examined on a phase or interference contrast microscope, when 200 sperm cells will be counted. Normal freshly collected buffalo semen usually has a widely varied pH (6.5 to 7.2). Although buffalo ejaculate has a lower concentration of sugars, the break down that occurs into the transformation of lactic acid can decrease the pH of semen. Meanwhile, the seasonal and climatic factors have a strong effect on the morphological and chemical seminal characteristics. In the temperate regions of the world it has been revealed that better quality semen is produced during the winter and spring and there is a deterioration in semen quality during the summer and autumn (2, 3). On the other hand, in the tropical regions the quality of semen is found to be good during the rainy season. In the warm and humid tropical Amazon region, the best time to work with semen freezing is between January and June (Vale, 1994). Buffaloes are more sensitive to heat stress, thus decline in semen quality is a common finding during the hot season of the year. The best manner to overcome the problem of semen quality deterioration due to thermal stress during summer is to sprinkle the animals with water during the hotter part of the day or allow the animals to wallow, protect the animals from radiation exchange and hot wind, and keep the animal in ventilated paddock. Free access of the animal to water and shadow is very important, since buffalo have a poorly-developed thermo-regulatory mechanism, thus they suffer a lot from thermal stress during summer weather.

Deep freezing of semen

The buffalo seminal characteristics that are correlated most highly with fertility and consequently with the possibility to use the semen, packaged and frozen are: volume, motility, concentration and morphology. Thus, before deciding to use the ejaculate for freezing technique, it is necessary that the males adapted for collection and ejaculates are normal concerning the seminal characteristics above mentioned. In tropical areas of the world, semen laboratories must have room temperature controlled at 20^{o} C and be equipped for evaluation, processing and freezing. The previous routine of semen examination and processing include different steps immediately after collection. This operation must be done as quickly as possible. Following the initial steps, the semen sample should be evaluated as described before and then put in a warm water bath at 30^{o} C and after five minutes it is diluted 1:1 with the extender fraction previously warmed at the same temperature. Thus it is important to add the diluent to the fresh semen as soon as possible after collection (Vale et al., 1991; Vale, 1994). After 15 minutes, another evaluation of the diluted semen must be done in order to assess the motility and vigor of the semen. Then the semen has obtained its final dilution according to the concentration and taken out at 20^{o} C temperature for 20 minutes when glycerolization occurs. During glycerolization period, the protection of spermatozoa against cold shock starts. Diluted semen have achieved good post-thaw motility and the least morphological and biochemical damage during freezing. Then, depending upon the freezing technique used, the semen is packed in straws. In our experience 0.25 ml German Mini-tubes or French 0.5 or 0.25 ml straws have been successfully used with good post-thaw motility and fertility. The final concentration recommended in our Laboratory is 40 x 10^6 sperm cells per dose, however depending of the semen quality and processing 20 x 10^6 sperm cells per dose can also be used successfully (Vale et al., 1991; Vale, 1994).

Buffalo production in Brazil

Beef production

One of the most important feature of buffalo is in the production of beef maintained on good management and nutritional conditions buffaloes in the Amazon basin had an average daily weight gain of 1,5 kg (Cabrera, 1988; Nascimento & Carvalho, 1993).

Cattle take 4-5 years to reach a suitable slaughter weight while buffaloes reach slaughter maturity in 2 years (Cockrill, 1977-1987).

Researchers from all over the world have done comparative feed trials and carcass evaluations between top quality crossbred beef cattle and random selected bulls and those investigations have testified to the advantage of buffalo performing favorably in the production of quality meat, Table 12, 13 and 14, (N.C.R. 1988). Currently buffalo is recognized as producing better meat than bovine. One of the known cost comparisons of producing buffalo and beef meat, was disclosed in the article ***Ancient water buffalo groomed to become wonder cow of the future***" written by Boyce Rensberger in the Washington Post, November 1986, where it was estimated that Florida beef cost about US $ 0.60 per live-weight pound to produce, while water buffalo meat cost US $ 0.49 per live weight pound. Although buffalo have shown a lower carcass production when compared to bovine or zebu at the same age, due to the higher weight of bones, horns and leather, they showed performances comparable or superior to bovine in feedlot operations, (Answer, 1988), Table 12.

Table 12. - Comparative production performances between buffalo and bovine species

Characteristic	Buffaloes	Bovine
•Native pasture support capacity	1 A.U./ 3.5 - 4.0 ha/year	1 A.U./2.5-3.0 ha/year
•Average calving	60 - 70%	40 - 50 %
•Dead by 1st year	5 - 6%	10 - 11%
•Dead by 1st - 2nd year	3 - 4%	6 - 7%
•Dead at adult age	1 - 2%	2 - 3%
•Culling	6%	9%
•Age at slaughter	2.0 - 3.0 years	3.5 - 5.0 years
•Weight at slaughter	300 - 400 kg	320 - 370 kg
•Milk production per lactation including cultivated pasture	1,000 - 1,400 kg	800 - 1,200 kg

Source: Nascimento & Moura Carvalho, (1993)

A.U.= animal unit=450 kg of body weight.

Table 13 - Average weight at birth and 24 months of buffaloes and bovine grazing on native pasture.

Breed	Number of observation	Birth weight (kg)	Number of observations	Weight at 24 mo.(kg)
•Mediterranean	71	36.80	19	368.95
•Carabao	32	36.75	10	322.70
•Jafarabadi	26	36.15	8	308.30
•Canchim	13	30.90	16	281.80
•Nelore	28	24.45	22	264.65

Source: Nascimento & Moura Carvalho, (1993) Summarizing it can be said that buffalo meat has 40% less cholesterol, 12% less fat, 55% less calories, 11% more protein and 10% more minerals compared to bovine, Table 14.

Table 14 - Comparative characteristics between buffalo and bovine meat

Characteristic	Buffalo	Bovine
•Calories. Kcal	131.00	289.00
•Protein (N x 6,25)	26.83	24.07
•Total fat (g)	1.80	20.69
•Fatty acids		
-Saturated total (g)	0.60	8.13
-Monosaturated (g)	0.53	9.06
-Polysaturated (g)	0.36	0.77
-Cholesterol (mg)	61.00	90.00
•Minerals		
-Total amount in (mg)		
Calcium, Iron. Magnesium, Phosphorus. Potassium. Sodium, Zinc, Copper and Manganese	641.80	583.70
•Vitamins		
-Ascorbic acid, Thiamin, Riboflavin. Niacin, Pentatonic acid, Vit. B_6, Folacine and Vit. B_{12}	20.95	18.52

Source: U.S. Department of Agriculture (1991)

Milk production

Wherever it is produced and available, buffalo milk is praised. When compared with the milk of other domestic species it is higher in nutritional value and is outstanding for the preparation of dairy products, Table 15. In many rural areas of the world, buffaloes offers a great possibility of farm labor, especially for small farming systems as a dairy animal. It is a common fact that the buffalo is a more efficient converter of roughage to milk than bovine. It requires 10,000 Kcal compared to 15,000 Kcal to produce a kilo of milk by a female buffalo and a cow respectively, in spite of more than twice the butter fat in buffalo milk compared to cow which requires, comparatively, a greater input (Sandhu, 1985).

Table 15 - Comparative characteristics and percentage constituents of buffalo, bovine and zebu milk.

	Fat	Protein(%)	Lactose	Total solids
•Buffalo	7.64	4.36	4.83	17.96
•Bovine	3.90	3.47	4.75	12.83
•Zebu	4.97	3.18	4.59	13.45

Source: Sandhu (1985)

Milk yield averages of 1,100 to 1,200 kg with 8.5 to 10 percent butter fat is commonly found among buffalo milk herds in the Amazon basin. In a survey performed in the Amazon region, analyzing the milk production between buffalo and zebu and zebu x crossed with *B. taurus*, buffaloes demonstrated to be better than cattle, Table 16.

Nowadays is possible to find out buffalo cows producing of more 4.000 kg per 300 days of lactation. In São Paulo state, there is currently a "boom" in milk production that is used in the manufacture of cheese, mainly "mozzarella and ricotta types". In comparison 10 kg of bovine milk but only 5 kg of buffalo milk is necessary to produce 1 kg of cheese.

Table 16 - A comparison between buffalo and zebu milk production in the Amazon region

Breed characteristic	Number of observations	Average milk production (kg)
•Murrah x Mediterranean	45	2.640
•Mediterranean	15	2.328
•Jersey-Sindi	9	1.990
•Sindi	17	1.635

Source: Nascimento & Moura Carvalho, (1993)

Draught animal production

In many countries of the world especially in Southeast Asia, buffaloes are the main source of power and labor work in the rural areas. At the moment, the buffalo has been recognized as an excellent option for moving power. However so far, it is not very much used as a draught animal in the other continents of the world. They can be used in the paddy fields, (rice-producing areas), for ploughing, pumping water, pressing sugar cane, harrowing and grading land and are very popular among peasant farmers in China, Vietnam, Thailand and other Asian countries. Its large feet, boxy hooves, coarse limbs and bulky body structures allow it to maintain a balanced traction in flooded soil and soft mud. This animal can be adapted particularly for forest work where, working in the shade long daily work hour can be maintained. An adult buffalo male can draw a carload of 500 kg for a distance of 25 km in a day. or will do all the work on 2 or 3 hectares of cultivated land. It can also be used to pull shallow-draft boats and in the Amazon it is ridden by barefoot peasants for herding other buffaloes and cattle. In Brazil EMBRAPA has conducted many experiments using buffaloes as a draught animal and has recommended its large multi purpose use mainly in flooded, muddy and forested areas and also the application of buffalo power in the pottery and brick-field industry of the Amazon valley (Pimentel & Reis, 1992).

Leather production

The thick tough hide is particularly suitable for the manufacture of certain types of heavy leather. It has been used for clothes, furniture, suitcases, car and airplane seats. In Germany a large enterprise, Jean Weipert-Traveller, specialized in travel equipment and fashion items, is using buffalo leather for its products. In Argentina and Brazil buffalo hides are used for clothing leathers, gloves, suede and upholstery. There is currently much interest in the buffalo hide, not only for local use but also for exportation to industrialized countries.

Buffalo Breeding in Brazil

It is reasonable to assume that the improvement of the buffalo in Brazil has been done without a centralized control and that this concern associated with the inbreeding problem has been a limitation for the further genetic development. Nevertheless, after 1980 the importation of semen from Italy and Bulgaria, through EMBRAPA (Brazilian Agricultural Research Corporation) was established leading to a new improvement of Brazilian buffaloes, mainly the Murrah, Mediterranean and Jafarabadi breeds, and a selection program has been developed to achieve a yearly genetic gain for traits like meat and milk. To apply this program cooperation between EMBRAPA West Amazon and CEBRAN (Center for Biotechnology of Animal Reproduction of the Federal University of Pará) was established. Thus a national system for testing and selection has been developed, which includes selection of the highest producing buffalo cows initially belonging to EMBRAPA, based on previous selection indexes already recorded. Furthermore, a testing and selection system for breeding yielding populations according to a crossing system, Figures 3 and 4, with the new generations having to pass an evaluation performance test for weight and milk production.

-Hereditary health, with special attention to a normal cariotype for the specie and absence of hereditary problems.
-Sexual health: every animal involved in the program must be checked for infectious and parasitic diseases, such as Brucellosis, Tuberculosis, Vibriosis, Trichomoniasis, Leptospirosis, IBR/IPV, BVD and Leukosis.
Moreover, for the sires involved in the program a Breeding Soundness Examination (BSE) is used for every sire involved in semen donation, according to the following criterion:
Andrological evaluation based on
-"Potentia coeundi", or in other words, the capacity to copulate
-"Potentia generandi" - the fertilization capacity - normal seminal position.

Deep freezing semen
In **CEBRAN**, the elite bull are kept for deep freezing semen and stored in straws, that are used in Brazil and for export. A program of progeny testing of buffaloes (**PRO-BUFALO**) using frozen semen of the bulls under field conditions has been introduced for use on different herds in Brazil through the following criterion:
A) Use by breeders that can purchase the semen from **EMBRAPA**.
B) Use by "Breeder-Partners", a selected group of farmers who receive the semen free of charge, as well as technical assistance and advise on the program, with the obligation of keeping **EMBRAPA** up to date on all information obtained in the AI program and on the progeny born. Thus, at this moment is carried out the **Progeny Test for Buffaloes of EMBRAPA - (PRO-BUFALO)**, which could be on a national scale because the environment involved in this test should vary as much as possible, this generating data for estimating performance indexes in production and reproduction of buffalo, culminating in the long desired genetic improvement of the regional and national herds, for the product will be available for all livestock farmers in general, Figure 3 and 4.

It should be remembered that, with the establishment of the **UFPA- CEBRAN** partnership, the research generates opportunities and material for participation in advance Biotechnological programs such as embryo transfers, FIV, superovulations among others.
The Amazonian Bank of Animal Germoplasma (**BAGAM**), implanted in Salvaterra county, on the island of Marajó, in collaboration with the UFPA. The Department of Genetic may also carry out a genetic characterization of animals using molecular markers with "*finger print*" techniques, an important tool utilized these days for determining paternity.
Results obtained so far with buffaloes of the Jaffarabadi, Murrah and Mediterranean breeds Marques et al., (1996) and Marques et al. (1997) are summarized as, Table 17. The average weight gain was 0.5 kg/day in almost all the categories of all the breeds under study.
The average secondary sex ratio, that is, the male :female ratio was 1.31:1 or 56,68% Jafarabadi males. For the Mediterranean breed, 86,11% on the births occurred in the rainy months and the sex ratio was 1:1,17 or 46,12% males, meanwhile for the Murrah breed the sex ratio was 1:1,5 or 48,78% males. The calving as in other genetic groups were concentrated in higher numbers or intensity in the drier months of the years. In the mixed breeds, the sex

Figure 3. Testing and selection system for breeding Mediterranean buffaloes by EMBRAPA

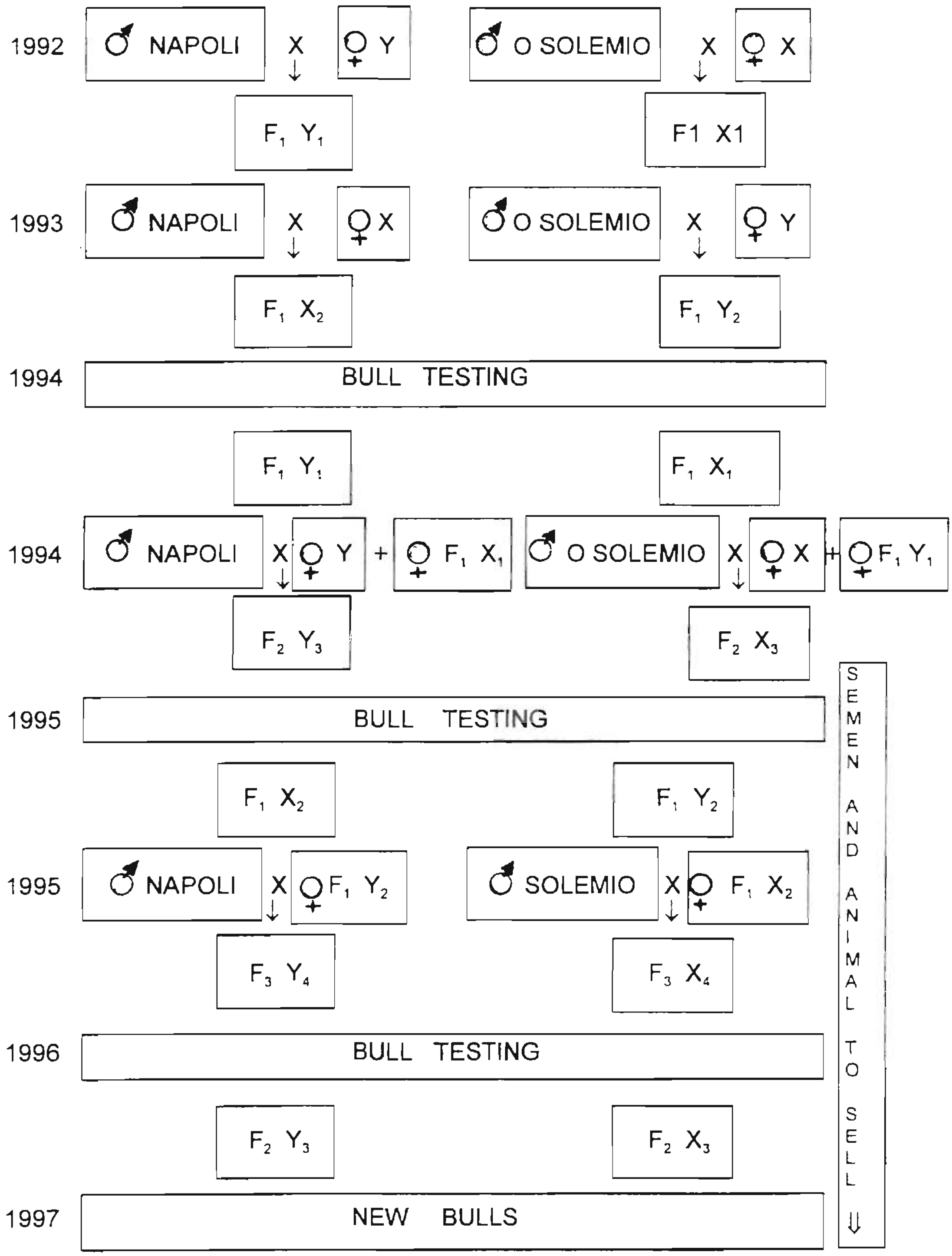
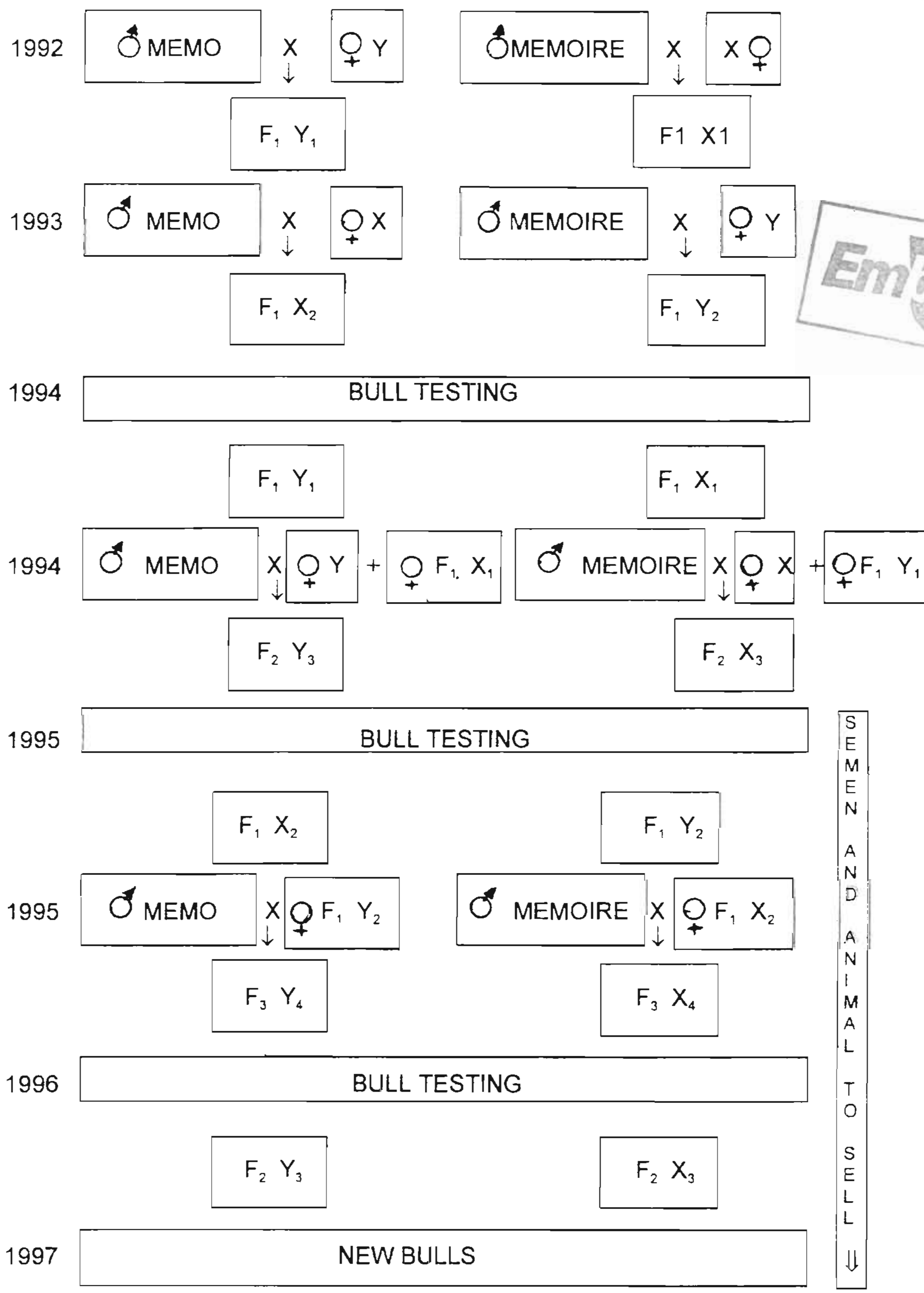


Figure 4. Testing and selection system for breeding Murrah buffaloes by EMBRAPA



ratio was, in general larger for males. The average productive live span varied from 3.06 years (n=4) in ½ Baio up to 6.60 years in ½ Carabao. With respect to the productive performance of the Mediterranean breed, progeny of imported bulls, the productive performances of three Mediterranean breed bulls were observed using average development data. Being two inseminated bulls of the three bulls using semen from imported bulls "Napoli" and "O Sole Mio", of Italian origin and the third of local origin, coming from Rio Grande do Sul. This national bull was put under natural mounting observation. The herd used, all of the Mediterranean breed, together with their young, were maintained in conditions, on the koronovia grass - *Brachiaria humidicola* with agro-industrial residual supplemented, basically, flour bran, at an average quantity of 2kg/day in milking cows and 1kg/day for calves up to 1 year of age.

The average development data, Table 17, shows the birth weight of the young imported bulls, males and females, whereas in the Table 18 shows the development data of the buffalo bulls in kilo.

The Table 19 shows the average daily weight at each age and it was observed that with the increase in age, the values of daily weight gains decreased in all the animals and that the young of the imported bulls always showed higher weight gains than the national bull. The animals assessed, 85 in number, in which 63 calves of the imported bulls and 22 from the national bull. It can be concluded that buffaloes presents, good productive and reproductive performances on conditions of native floodplains pasture of the Amazon which serve as a great alternative in the supply of quality proteins for the populations of the region. In general, all the genetic groups shows great capacity for adapting to the adverse conditions of the Amazon.

Table 17 - Productive performance data of buffaloes in the Amazon Region.

Breeds and crosses	BW/n	PF/n	AFC/n	CI/n	SP/n	Longevity/years/n
Me	32.07 ±0.20 (950)	498.08 ±86.80 (13)	1076.65 ±90.98 (199)	386.66 ±3.97* (583)	72.94 ±2.79* (532)	7.79 (1439)
Mu	33.78 ±3.44 (192)	564.07 ±69.44 (14)	1051.71 ±86.99 (35)	383.72 ±4.66 (124)	68.30 ±3.08 (110)	6.86 (328)
½ Mu	32.50 ±2.49 (170)	380.0 (1)	1073.26 ±135.23 (19)	387.69 ±30.33 (13)	74.16 ±28.76 (12)	5.19 (79)
½ Me	32.77 ±2.96 (76)	539.33 ±138.55 (3)	1055.80 ±119.94 (55)	396.42 ±30.62 (43)	78.05 ±25.92 (36)	4.63 (163)
½ Ca	32.87 ±2.41 (8)	_	_	395.0 (1)	85.0 (1)	6.60 (4)
½ Ba	_	_	820.0 ±21.21 (2)	_	_	3.06 (4)
Crosses	32.80 ±0.50* (525)	481.58 ±55.43 (12)	1,102.0 ±116.15 (28)	395.60 ±31.10 (41)	74.85 ±23.52 (34)	5.77 (174)

Me-Mediterranean;Mu-Murrah;Ca-Carabao e Ba-Baio / * - Erro.

Table 18 - Development data of the buffalo bulls in kilograme.

Ages	Bulls						% gain imported
	Napoli		O sole Mio		Host		
	M	F	M	F	M	F	
Born	39.19	36.66	39.20	38.00	35.94	34.15	9.18
30 days	70.48	66.23	69.46	67.69	67.58	59.69	7.59
90 days	123.14	116.38	122.71	120.39	114.81	105.21	9.67
180 days	194.61	188.61	193.10	185.45	176.35	160.82	12.97
1,0 year	292.58	285.18	273.66	254.50	241.04	224.29	17.68
1,5 year	405.34	365.78	379.11	346.42	334.10	291.35	19.65
2,0 years	483.30	437.26	456.00	429.12	441.04	385.53	9.22
2.5 years	509.2	467.72	502.00	478.68	489.42	425.57	6.97
3,0 years	590.20	542.50	594.65	551.94	586.83	455.20	9.37
Average							11.37

Fonte: Marques et al. (1996)

Table 19 - Daily weight gain of buffaloes in kilograme, 1995.

Age	Bulls					
	Napoli		O Sole Mio		Host	
	M	F	M	F	M	F
30 days	1.04	0.99	1.05	0.99	1.05	0.85
90 days	0.93	0.89	0.93	0.92	0.88	0.79
180 days	0.86	0.84	0.85	0.82	0.78	0.70
1 year	0.69	0.68	0.64	0.59	0.56	0.52
1.5 year	0.67	0.60	0.62	0.56	0.55	0.47
2 year	0.60	0.55	0.57	0.54	0.55	0.48
2.5 years	0.51	0.47	0.51	0.48	0.50	0.38
3 years	0.50	0.46	0.51	0.47	0.50	0.38
Average						**11.37**

Fonte:Marques et al. (1996).

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