Buffalo Newsletter

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BULLETIN OF THE FAO INTER-REGIONAL COOPERATIVE RESEARCH NETWORK ON BUFFALO AND OF THE INTERNATIONAL BUFFALO FEDERATION - INCLUDES SHORT COMMUNICATIONS, RESEARCH PAPERS, TECHNICAL NOTES, ONGOING RESEARCHES

FAO – IBF – ICAR
BUFFALO REPRODUCTION SATELLITE MEETING

July 12-13, 2008 – Budapest, Hungary

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IMPORTANT ANNOUNCEMENT

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The FAO IBF ICAR Buffalo Reproduction Satellite Meeting was held in Budapest (Hungary) on 12-13 July 2008, sponsored by FAO/ESCORENA (The European System of Cooperative Research Networks in Agriculture, Budapest) by IBF (The International Buffalo Federation, Monterotondo, Rome, Italy), by C.R.A. (Agriculture Research Council) Animal Production Research Centre, Monterotondo, Rome, by ISPAAM C.N.R. (Institute of Animal Production in Mediterranean Area of National Research Council, Napoli, Italy), by COFA (Cooperative Artificial Fecondation, Cremona, Italy), by ICAR (16th International Congress of Animal Reproduction, Budapest).

The meeting was held in the FAO Headquarters in Benczur road and the welcome and presentation was made at 15,00 on July 12 by FAO staff: Maria Kadlecikova, Regional Representative for Europe and Central Asia, Aleksej Tarasjev, Agricultural Research and Biotechnology Officer, Michel Demes, Information and Knowledge Management Officer.

Prof. Antonio Borghese presented the activities of the FAO Inter-Regional Cooperative research Network on Buffalo and of the International Buffalo Federation: World Buffalo Congresses, FAO meetings, International Projects, links between scientists and International Organizations, promotion of information and research, publishing of the Buffalo Newsletter.

After that the Session 1 started on "Male reproductive physiology and endocrinology – Genetics of reproduction" with a main paper by prof. William Vale and 4 communications reported in following. The meeting carried on in the Paprika restaurant where typical dishes were offered to the glad participants.

The day after, July 13, 2008, the Session 2 was opened on "Artificial insemination and embryo transfer, gestation and embryonic mortality, mammary gland and milk production", where the following 3 main papers and 3 communications were presented and discussed.

Finally prof. William Vale showed a video on Genetic selection of Murrah buffaloes in Brazil.

The meeting finished with a general, very vivacious discussion and some conclusive remarks: the promotion and extension of the FAO website of the Buffalo Network in the ESCORENA organization to promote information and links, the possibility to organize a new meeting of the Buffalo network with the cooperation of the FAO office in Ankara, Turkey on the buffalo management in development countries.

INTERNATIONAL BUFFALO FEDERATION
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ESCORENA

INHERITANCE CAUSES OF BUFFALO BULLS INFERTILITY IN BRAZIL

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ABSTRACT

Although changes in environment and management have been primarily implicated in the in reproductive efficiency decline of buffaloes in Brazil during the past two decades infertility of hereditary origin of the male and female has been observed among the Brazilian buffalo herds and it is important to pay attention to the male fertility which must not be overlooked. Recent attempts were done to established measures for scrotal circumference in Murrah buffalo bulls in Brazil with the aim to standardize this important pattern and improve the fertility potential of an artificial insemination (AI) in Brazil and Latin America. Additional semen quality attributes associated with fertility may provide more accurate methods to predict, manage, and select buffalo bulls for AI. Because the values of most known semen quality traits are highly correlated, any new technology must be considered with respect to the additive benefit imparted compared to existing methodologies (improved fertility prediction or economic utility of implementation). The presence of different forms of hereditary disturbances in the genital system of the male buffaloes has caused problems in the expansion of some breeds in Brazil and Latin America. It is necessary to perform the use of the Breeding Soundness Examination (BSE) in buffalo males as a form to predict the increase of the herd's fertility and avoid the presence of animals with unfavorable genes and the spread of these pathologic characteristics. Unfortunately, the association between semen quality and fertility is usually limited by the absence of accuracy of the fertility estimate in the male buffaloes used at regional level.

Keywords: Amazon, buffalo, fertility, infertility, inheritance disturbances

INTRODUCTION

Stirring both popular and scientific curiosity, the study of breeding troubles in the domestic species must be guided to the causes of infertility. Thus it is particularly important to try to find out which forms of sterility of infertility are caused by hereditary factors and which forms are caused by the environmental factors. From the breeding point of view this question is of fundamental importance not only for the technicians but also for the farmers. However, in the majority of the cases farmers are not interested on this concern due the fact that the occurrence of such infertility problems depreciate economically the herd and push the problem in the majority of cases for drastic measures regarding the discharge of some animals inside the herd affected. Lagerlöf (1934) reported in the early of the last century that in cattle the sexual health control tackled the question of certain causes of infertility. This aspect was possible to be implemented due the rapid development of artificial insemination which produced better possibility than before for the study of the sexual patho-physiology of the male and female of domestic animals to obtaining an accurate estimation of the fertility rates inside of different herds. After the WW II many reporters in different part of the world, began to take a serious interest in these problems that affected the farm animals mainly cattle *(Spriggs, 1946; Gillmore, 1949; Götze, 1949; Bane, 1952; Rollinson, 1950; Nordlund, 56; Johansson, 1960)*.

Based in these and other reports Lagerlöf *(1951)* proposed a scheme to identify the causes of infertility in the domestic animals (as follow in page 4).

Furthermore in the domestic buffalo many reporters in different part of the world, began to point the existence of infertility problems particularly in the clinical literature describing and illustrated gross reproductive abnormalities of various types *(Hafez, 1954; Maurya et.al., 1969; Chaudhry et. al. (1978); Kaikini and Patil (1978), Chaudhuri et al. (1982) and Rao Ramamohana (1984)*.

For Basrur; Basrur *(2004)* many genetic and congenital forms of infertility can be framed inside the study of hereditary pathology which deals with different forms of abnormal development in the phenotype and genotype of

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CAUSES OF INFERTTILY

I. CONGENITAL AND HERIDITARY FACTORS

A. Developmental anomalies

1. Anomalies in development or descent of gonads.
2. Anomalies in development of embryonic duct systems.

B. Certain lethal factors

C. Predisposition to endocrine Distrurbances

1. Disturbances in endocrine glands function (hormone production)
2. Disturbances in spermatogenesis.
3. Disturbances in ovulation and nidation.

II. ACQUIRED OR ENVIROMENTAL FACTORS

A. General diseases

B. Diseases and lesions localized in the reproductive organs mostly due to infection:

1. Coital diseases
2. Puerperal.

C. Malnutrition

1. Overfeeding.
2. Underfeeding.
3. Mineral deficiencies.
4. Vitamin deficiencies.

III. COMBINATION OF BOTH

the domestic animals characterized by errors of metabolism, congenital defects and genetic of infertility caused by deleterious or unfavorable genes which affect indistinctly man and domestic animals. Many factors which directly are associated with inheritance pathology in buffaloes have been described the in the international literature *(Vale, 2005)*.
In Brazil in spite of the huge development of buffalo husbandry, this species is raised in the whole country where four breeds Murrah, Mediterranean, Jafarabadi and the Swamp type called Carabao were introduced more than a century ago. For the origin of these breeds it has been introduced a reduced number of animals in the whole Brazil, although in the last two decades also frozen semen from other countries was imported. Even though the origin of Murrah breed was based in animals Pure Imported of Origin (POI) since only 11 female animals and four males imported from India gave origin the formation of this breed in Brazil *(ABCB, 1986)*. Not in the least it is expected to be found seemingly endless variety of forms of hereditary problems which have continued to drawn attention as a cause of reproductive failures in the buffalo herds, some imposed by inbreeding practices, and others caused at random; however, the majority occasioned by the intensive intentional inbreeding with the aim of genetic improvement *(Vale, 2005)*. The role of the male on the reproductive efficiency of the buffalo herd is well known in the other domestic species, however in buffalo there is a lack in the literature. Therefore, the use of basic techniques such as the BSE to screen the potential reproductive capacity of the male has proven useful in detecting males with potentially low fertility. Thus, the purpose of this paper is to describe new forms of inherited causes of infertility found in buffaloes herds raised in Brazil.

HEREDITARY ABNORMALITIES IN THE MALE GENITAL SYSTEM

Abnormal formation of scrotal sac.
The normal scrotal sac in the male buffalo looks like as in the other ruminants, with the two normally suspended testis hanging in the scrotum so that the caput, body and tail of the epididymis can easily be palpated. Further the dorso-lateral wall did not form as in the bovine the typical neck especially in the young animals. It has been frequently observed in the Murrah breed in Brazil different forms of scrotum abnormalities likely twisted or in abnormal formation of the scrotal wall and septum dividing the scrotal pouch or less extend abbreviated caudal scrotal wall, so that the testis are unilaterally or bilaterally held in a near horizontal position. Such abnormalities sometimes affect more than 10 per cent of the males in some herds and it seems to be linked to the high inbreeding that has been used in the breeding programs adopted by the farmers. In a study conducted in 123 Murrah buffalo bulls which were submitted to Breeding Soundness Examination (BSE) with commercial purpose, Vale et al. *(unpublished data)* found 10.8 per cent of the animals submitted to the clinical examination presenting different forms of middle or severe abnormalities in the scrotal pouch. Within limits, the degree and significance the abnormal formation of the scrotal sac in some herds of Murrah breed in Brazil and Latin America affect not only the morphology of the external genital system but also play a big role

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in the degeneration of the testis and deterioration of the seminal picture in the animals affected which have a tendency to testicular degeneration and fibrosis in both gonads and consequently causing infertility.

Cryptorchidism.
This is another inherited condition characterized by incomplete descent of the testes either unilaterally or bilaterally *(McEntee, 1990)*. This condition has been found in buffalo bulls in Brazil. There is an indication in other species that a dominant recessive gene is associated with this anomaly. Probably, it can be also caused by the progressive inbreeding used in some buffalo herds in Brazil and South America.

Testicular hypoplasia.
This is a genetic problem which occurs in all species of domestic animals, and has been very well documented for the bovine by Settergren *(1985)* and McEntee *(1990)*. The gonadal hypoplasia is a congenital, mostly hereditary condition which can be unilateral or bilateral and total or partial. Affect both male and female *(Lagerlöf, 1934, 1951)*. In the buffalo species there are reports about this disease from different countries *(Kaikini;Patil,1978; Chaudhuri et al., 1982; Rao Ramamohana, 1984; Vale et al., 1988)*.
Vale et. al., *(1980)* reported a case in one buffalo male where the left testis was smaller than the right one, where no germinal cell were inside the seminiferous tubules however Sertoli cells were present between the tubules; so, this case was similar to the typical case of total hypoplasia classic type as described in bovine by *(Lagerlöf, 1934)*.
Moreover Ohashi et. al., *(1988)* found a case of bilateral partial hypoplasia in a Mediterranean buffalo bull, aged 3 years, presenting symmetrical testis 7.5 cm x 3.5 cm lower than the normal average observed by Vale et. al., *(1981)* for regional buffaloes. The scrotal circumference was smaller than the normal and concentration was low but the ejaculates showed high percentage of sperm abnormalities, such as abaxial and underdeveloped type, associated with abnormal germinal cells, mainly spermatids. The figures of this case which is in accordance with the findings report by *(Kaikini; Patil, 1978)*. Again Ohashi et al., *(1995)* studying 319 genital systems of crossbred buffaloes collected from a slaughterhouse in Belem city, northern Brazil found 22 cases (6.89%) of abnormal testicular development. In two cases it was found unilateral hypoplasia in the left testis with the both gonads very small with firm consistence. Histologicaly, the right testis were normal, however on the left, in spite of the fact that it was very small in size, in the majority of the seminiferous tubules the spermatogenic process reached the phase of round and elongating spermatids which indicate that the process was active. Also it was found cases of abnormal testicular development characterized by gonadal aplasia. Through the histological examination small agglomerate of tubular structures, resembling sex cords were found, surrounded by a thick layer of connective tissue. The epididymis was hypertrophied and bigger than normal adjacent one, covering the small gonad completely. Furthermore, chromosomal analysis performed in four animals showed that two animals had the karyotype 2n=50 XY (normal) but in two other cases it was 2n=49, XY (4p,9q) which was characteristic of hybrid animals.
Recently, Vale et al., *(unpublished data)* found many cases of uni and bi-lateral hypoplasia in buffaloes of the Murrah breed caused probably by inbreeding practices.
The testicular hypoplasia can affect one or both testicles with the hypoplastic testicle being always notably smaller that the normally developed one and usually the affected testis to not sink so far down to the bottom of the scrotal pouch. The scrotal circumference (SC) is small and in the bilateral case both testicles are too small in relation with the normal animals. Throughout the routine breeding soundness examination (BSE) accomplished with the clinical and spermiogramme examination the buffalo males were classified as unsound or sound for breeding.
In a study conducted on 123 Murrah buffalo bulls age 20 to 50 months which were submitted to a breeding soundness examination (BSE), *Table 1*, for commercial purpose, it was found that the majority of animals were from the pure line of POI Murrah breed and evidences confirmed that many of them were submitted to close intrabreeding or crossbreeding showing a high homozygotic pattern which may have a direct or indirect effect on the fertility. The incidence of testicular hypoplasia, determined based on visual appraisal of the testes at the time of SC measurement and confirmed through the BSE were eight cases (5.69%) of bilateral partial and two cases (1.62%) of uni-lateral total and one case (0.81%) of bi-lateral total hypoplasia in an overall presentation of 11 males (8.94%) with the incidence of this diseases *(Vale et al. no published data)*. No bulls

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Table 1. Clinical characteristic and seminal picture of 11 Murrah buffalo bulls submitted to Breeding Soundness Examination (BSE) and found with gonadal hypoplasia.

N.	AGE	TESTICLE		SC	VOL	CONC.	MOT.	VIGOR	SPERM PATHOLOGY %	
	Mo.	R	L		ml	10^3/mm	%	%	Major	Minor
01	55	P	P*	30	3.5±2.1	75±34.4	40	1	26.5	18.9
02	26	P	H	19	1.9±1.1	44±29.4	30	1	33.4	12.2
03	33	H	H	16	-	-	-	-	-	-
04	32	P	N**	20	2.8±1.6	61±39.8	30	1	29.8	14.3
05	29	P	N	21	2.6±1.5	56±39.2	60	1	31.1	19.2
06	33	P	P	24	2.4±0.9	78±52.2	40	1	33.9	16.5
07	40	P	P	26	2.9±1.2	68±53.5	50	2	26.9	18.3
08	28	P	P*	19	1.6±0.8	51±66.4	20	1	28.6	23.4
09	30	P	P**	20	2.6±1.9	58±42.3	30	1	28.4	15.1
10	38	P	P	25	4.1±2.8	39±39.5	40	1	36.5	26.4
11	44	P	H	28	3.6±2.2	72±52.7	30	1	25.6	19.4

*H=totally hypoplastic P=partially hypoplastic N=normal *=full brother ** half brothers*

failed the BSE due to physical soundness problems of the feet and legs, poor body condition, or disease. The testis consistence was either soft and flabby or hard indurated and fibrotic and decreased in size. Males which are severely affected are nearly sterile but an occasional conception may occur in female bred to them. The semen picture showed a low concentration of spermatozoa usually less than 75.000 /mm3, low motility, wave motion and vigor with many abnormal spermatozoa and the presence of few giant cells. The affected testis are 1/3 to 2/3 of the normal size but are usually firmer on palpation. Microscopic examination showed that the majority of the seminiferous tubules were underdeveloped. In the present study depending upon the degree of hypoplasia, affected animals had a small firm epididymis especially in the tail region, indicating reduced spermatogenesis.

Table 2. Seminal picture of eight Murrah buffalo bulls with bi-lateral partial classic gonadal hypoplasia.

Volume (ml)	x=3.62±1.36
Concentration x10^3/mm^3	x=64.5±11.26
Motility %	0-20=4; 25-40=2;45-60=2.
Vigor	1-2
Wave motion	0=4;+=3;++=1
Sperm pathology (%)	
-Head	x=28.16±11.45
-Middle piece	x=4.55±3.07
-Tail	x=10.96±8.52
-CPD	x=15.40±22.61
-CDD	x=1.26±2.11
-Acrossome	x=1.16±1.9

Table 3. Seminal picture of two Murrah buffalo bulls with uni-lateral total gonadal hypoplasia in an average of 10 collections.

Volume (ml)	x=2.71±1.7
Concentration x10^3/mm^3	x=59.3±40.1
Motility %	0-30
Vigor	1
Wave motion	0-20
Sperm pathology (%)	
-Head	x=30.6±24.1
-Middle piece	x=6.5±3.2
-Tail	x=12.4±6.2
-CPD	x=17.1±9.1
-CDD	x=2.2±0.9
-Acrossome	x=2.1±1.9

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MALFORMATION IN THE EPIDIDYMIS AND EPIDIDYMAL DYSFUNCTION

Arrested development of mesonephric ducts - Spermatic granuloma.

The retention of spermatozoa in the epididymis can be caused by blind efferent ducts or segmental plasia. This condition, named spermatic granuloma, has been rarely reported in the buffalo bull *(Maurya et.al., 1969)*, however it was observed in Brazil by Vale et al. *(1980)*. Recently Vale et al., *(2002)* found a case of a Murrah buffalo bull, born through artificial insemination of semen imported from Bulgaria and a native Brazilian Murrah female, age 21 months, raised in a milk herd. During his rearing period he was clinically health. At 15 months of age ,he was first clinically examined, he showed a normal scrotal circumference of 31 cm, and a normal shap for the both testis and epididymis; however by the rectal examination of internal genitalia, it was observed an abnormal development of the ampoules of deferent ducts. No abnormality was found on examination of external genital organs, except an abnormal protuberance in the tail of the left epididymis which by palpation showed to be hard. Semen collection through artificial vagina was tried, however, every effort was hopeless. As the bull could not be used for breeding and his fertility was unknown and also due the increase of the clinical abnormalities in the ampoules and in the tail of the epididymis, it was decided to slaughter the animal. The post mortem examination the tail of the left epididymis shown a cystic dilatation in the epididymal duct with accumulation of sperm in the cyst cavity (spermatocele) which after cutting a yellowish secretion flow from the cystic cavity. The internal genitalia showed the presence of an accessory ampoule and in the normal left ampoule a segmental aplasia in medial segment with the cavity, full of yellowish secretion. The other segments of the internal genitalia were normal. This case was manifested by an arrested development of portion of mesonephric ducts which, embryologically, give rise to the ampulla of deferent ducts, epididymis. aplasia in the epididymis is a common finding in the different domestic species, however aplasia of accessory deferent ducts has been reported as rare. For McEntee *(1990)* such malformation may be a congenital defect rather than an acquired lesion. Other possible causes for variability in expression of the defect might include additional gene involvement or the existence of modifying factors related to so-called “penetrance” and “expressivity” *(Basrur, 1980;1988)*. Furthermore, it is important to stress that such anomalies in the majority of the cases are accomplished with other systemic abnormalities in different systems of the animal organism, as in the case of the white heifer diseases, described in the British Shorthorn breed *(Spriggs, 1946)* or associated with depigmentation in some part of the body, showing some white spots in the head as well as in the eyes. Although results in the present case did not provide adequate data for firm opinions, it seems very possible that the problem of arrest development in both mesonephric and paramesonephric ducts exists in the Brazilian Murrah breed group as well as in the Bulgarian sires which were used for deep freezing program for genetic improvement. Efforts must be done for the identification of cases like here described. the sequential increase in the number of cases of arrest development of mesonephric ducts may be possible if conditions such this is overlooked. It is important that Veterinarians, geneticist, animal scientists and breeders pay attention for cases like this here presented due the importance for the future fertility of the herds, mainly those that are using artificial insemination as a tool for genetic improvement for Murrah herds in Brazil and Latin America.

Epididymal dysfunction.

Epididiymal dysfunction has been described in the Bos taurus and Bos indicus by Gustafsson *(1966)* and Vale Filho et.al. *(1978)*. In the water buffalo it was first described by Ohashi et. al., *(1986)* in top-level Murrah bull of 42 months, 600 kg of body weigth, good libido, symmetrical testes (10.5 cm x 6.5) and 33 cm scrotal circumference. Both testes and epididymis were normal, the tail of epididymis was prominent, indicating a good reserve capacity. The animal was used for semen collection for 9 months, in artificial vaginal, twice a week; the following average values were obtained: volume= 4.22mL; wave motion= 0; motility= 0-10%.; head abnormalities= 7.5%;.tail abnormalities= 56.5%. Throughout two exhaustion tests at an interval of two weeks, five and six collections were obtained in the artificial vaginal in a period of 70 to 90 minutes. As the collections were performed, the level of tail abnormalities decreased while sperm motility increased, in both test, with the typical feature of epididymal dysfunction as described by Gustafsson *(1966)*; Vale Filho *(1978)*.

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Unspecific cases of sperm abnormalities.
Unspecific sterilizing and hereditary defect on the middle piece and tail associated with reduced fertility has been documented in the literature affecting bovine and equine *(Blom, 1959; 1972)*. Such abnormalities referred as corkscrew, dag defect, stumpf tail defect affect the middle piece development or causing an irregular distribution of mitochondrial sheath and the high presence bent the tail in the ejaculates.
Recently Vale; Ribeiro *(2007)* found in young Murrah bulls belonged to elite herds in northern Brazil a new sterilizing and hereditary form of sperm abnormality described by Blom *(1972)* as dag defect. The term dag defect was coined in the Danish Jersey breed when a young bull named Dag and one year latter in his younger full brother showed a large number of sperm abnormalities characterized by coiled and folded tails *(Blom, 1972)*. Both bulls showed low fertility and a very peculiar sperm pathology. Danish workers conducted further studies and tracing back to a common ancestor born in 1934 and found 15 related bulls. All the animals affected had a low initial spermatozoa motility of 10 to 15 per cent and very poor fertility. These bulls had about 40 to 50 per cent of their sperm cells with strongly coiled, folded or split tails. The fibbers in the axial filament were normal in the testis but abnormal when the cells reached the cauda of epididymis. This defect was shown to be due to an autosomal recessive factor *(Blom, 1959, 1972)*. Afterwards this abnormality was described in other countries in Bos taurus and Bos indicus including in Brazil. In buffaloes, Vale; Ribeiro *(2007)* found many cases of this problem associated with inbreeding practices. In some buffalo males the presence of 20-30 per cent of sperm cells with dag defect has been commonly observed.

Seminal vesicles and ampoules.
Arrested development of the seminal vesicles has been most studied in the bull. It is mainly caused by segmental aplasia or hypoplasia of the mesonephric ducts when malformation occurs in the sex organs derived from this kind of ducts *(McEntee, 1990)*. Abnormalities in the seminal vesicles have been report as to be rare by Kaikini;Patil *(1978)* and Chaudhuri et. al., *(1983)*. In our observations with slaughter-house material, we found two cases, one unilateral and the other bilateral, of segmental aplasia in the seminal vesicle and one case in the ampullae.

CONCLUSION AND RECOMMENDATIONS

Inherent variabilities in the composition of the different buffalo breeds raised in Brazil showed that samples and the efficacy of the detection and appraisal of inheritance causes of buffalo bulls infertility are present and can not be overlooked. Although most of the material herein presented and discussed is from Murrah breed, it is possible that the same type of fertility impairment can occur in the other breeds. Further investigations should focus on the other breeds and become more intensive in the Murrah breed. It is necessary the introduction of semen from foreigner buffalo males to refresh the blood of the different herds. Studies must be conducted with the aim to afford a more objective evaluation of each abnormality based on the ultimately endpoint on the herds fertility based on the breeding soundness examination of each buffalo bull used in the reproductive programs.

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CYTOFLUORIMETRIC DETERMINATION OF VIABILITY, CHROMATIN STABILITY, MITOCHONDRIAL FUNCTION, AND ACROSOME REACTION IN FROZEN THAWED BUFFALO *(Bubalus bubalis)* SPERMATOZOA

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ABSTRACT

Flow cytometry (FC) is a useful tool for quick, multi-parametric and objective determination of sperm quality. To date, simultaneous assessment of different quality parameters has not been reported in the buffalo. Frozen-thawed spermatozoa from two sires were analyzed by FC. Sperm viability, chromatin integrity (SCSA) and mitochondrial function were evaluated after thawing. Acrosome status was analyzed immediately after thawing and after 4 hrs in TALP medium in presence/absence of calcium ionophore (A23187). The mean viability was 51 ± 15 % (mean ± SD). Concerning SCSA, the DNA Fragmentation Index (DFI) parameters were (mean ± SD): 118 ± 5 for DFI, 22 ± 5 for *X* DFI and 65 ± 6 for SD DFI. The percentage of spermatozoa with high mitochondrial membrane potential (active mitochondria) was 56 ± 10. After thawing, the percentage of acrosome-reacted spermatozoa (ARS) was 8 ± 2. After 4 hrs, the ARS percentages were 16 ± 3 and 50 ± 6 in the absence and presence of 1 μM Ca^{2+} ionophore A23187, respectively. This is the first report on cytofluorimetric evaluation of acrosome reaction and mitochondrial function in frozen-thawed buffalo spermatozoa.

INTRODUCTION

Accurate evaluation of fertility of bulls used for artificial insemination (AI) is of utmost importance since a single ejaculate provides several insemination doses and influences the reproductive potential of a herd [1]. Although AI has been practised for the past 50 years in buffalo species, fertility rate with this technology is low and unpredictable in buffaloes [2]. Several methods have been developed over last years for laboratory evaluation of semen quality and fertility. However, the most reliable approach to predict fertility potential of semen sample is to evaluate different attributes on individual sperm [3]. To date, simultaneous assessment of different buffalo sperm quality parameters has not been reported.
The aim of this study was to assess the frozen buffalo sperm quality using rapid and objective methodologies able to evaluate different sperm cell parameters. In particular, on frozen thawed sperm samples collected from two buffalo sires, viability, stability of sperm chromatin structure (SCSA), mitochondrial membrane potential and acrosome reaction were assessed by flow cytometry.

MATERIALS AND METHODS

Frozen semen straws of two Italian Mediterranean buffalo sires, routinely used in AI programs, were analyzed by flow cytometry FACSCalibur (BD, Milan, Italy) equipped with a 15 mW air-cooled Argon laser.

Sperm viability

Viable spermatozoa, are defined as cells with intact plasma membrane which prevent the entry of propidium iodide (PI). Sperm cells were stained with PI (2 μg/mL), excited at 488 nm, and read with 650/13 nm bandpass emission filter (FL-3) using a logarithmic histogram. Cell viability was expressed as the percentage of PI-negative cells *(see Fig. 1A)*.

Sperm Chromatin Structure Assay (SCSA)

The SCSA was performed by using the metachromatic properties of the fluorescent acridine orange (Sigma), related to DNA structure [4]. Acridine orange emits green or red fluorescence when it intercalates to double-stranded (native) or single-stranded (damaged) DNA, respectively. The used protocol was previously described by Benzoni *et al* [5]. The instability of sperm structure chromatin was measured using different parameters of the DNA Fragmentation Index (DFI) as the mean (*X*), the percentage (%) and the standard

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deviation (SD) DFI *(see Fig. 1B)*.

Mitochondrial function assessment

Mitochondrial membrane potential ($\Delta\psi m$) was measured by JC-1 (Molecular Probe), a molecule that can exist in monomer form, with $\Delta\psi m^{low}$ (green fluorescence) and in aggregate form with $\Delta\psi m^{high}$ (orange fluorescence). Emission filters of 535 nm and 595 nm were used to measure green (FL1) and orange (FL2) fluorescence, respectively and the percentage of cells with $\Delta\psi m^{high}$ was assessed *(Fig. 1C)*.

Acrosome reaction assessment

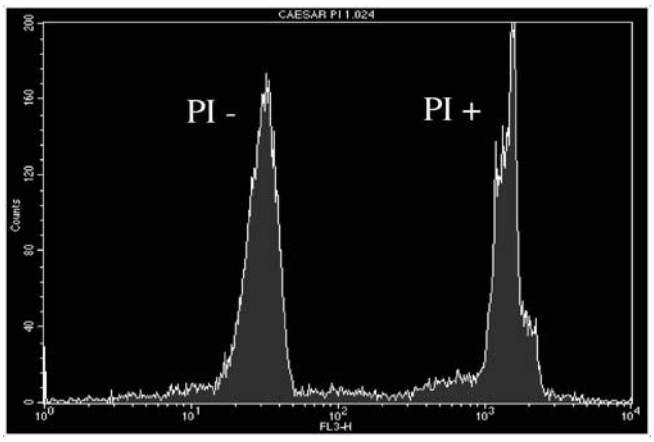
The acrosome status of sperm cells was examined by staining with PNA-FITC (EY Laboratories Inc.) and following the protocol described by Rathi *et al* [6]. Acrosomal status was assessed immediately after thawing and after 4 hrs in TALP medium in presence or absence of 1 μM Ca^{2+} ionophore A23187. On FL1/FL3 dot-plot *(see Fig. 1D)*, the percentage of acrosome-reacted spermatozoa (live and dead) was evaluated as the population with high green fluorescence (FL1).

RESULTS AND DISCUSSION

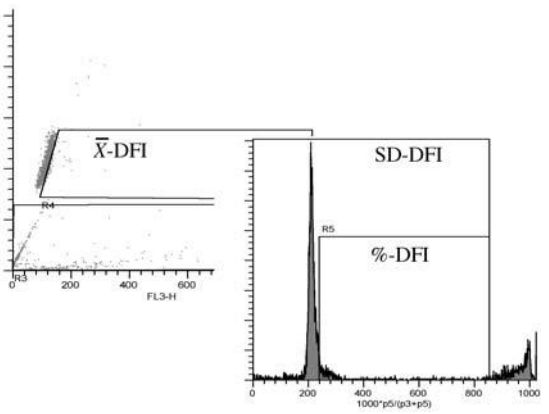
The mean viability was of 51 ± 15 % (mean ± SD) and ranged from 40 to 62 %. Concerning SCSA, the DFI parameter ranged between 105 and 127 with a mean of 118 ± 5. The %DFI parameter ranged between 16 and 36 with a mean of 22 ± 5. The SDDFI parameter ranged between 56 and 78 with a mean of 65 ± 6. The percentage of cells with $\Delta\psi m^{high}$ was 56 ± 10 and ranged from 49 to 63. After thawing, the percentage of acrosome-reacted live and dead spermatozoa (ARL and ARD, respectively) was 0.5 and 7.5 ± 2. After 4 hrs in TALP medium, the ARL and ARD percentages were 2 ± 1.4 and 14 ± 1.4, respectively. At the same time, in the presence

Figure 1. Representative analysis of viability, chromatin integrity (SCSA), mitochondrial function and acrosome reaction of frozen-thawed buffalo spermatozoa by using flow cytometry.

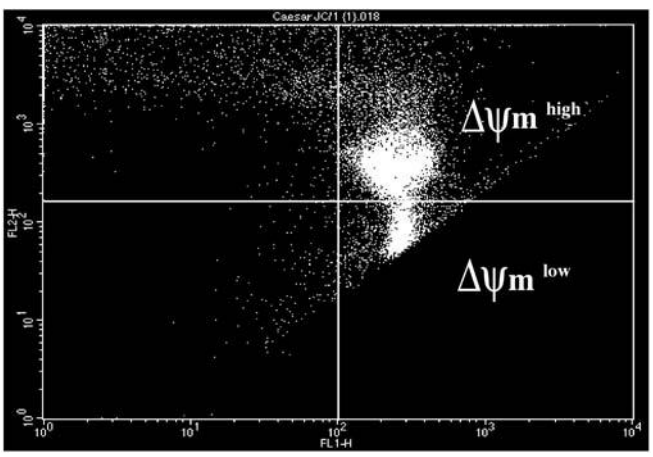
A: Viability



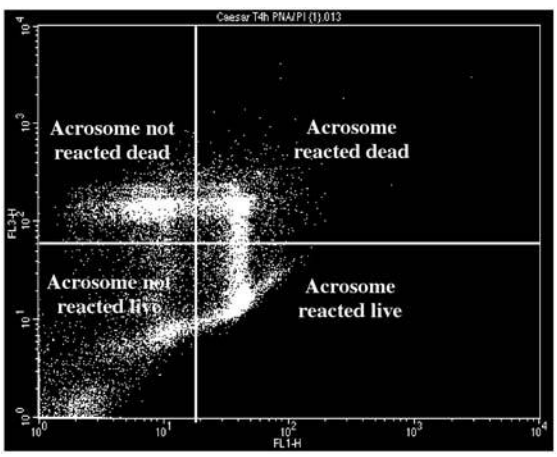
B: SCSA



C: Mitochondrial function



D: Acrosome reaction



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of 1 μM Ca^{2+} ionophore A23187, the ARL and ARD percentages were 22 ± 5.6 and 28, respectively.
This is the first report which evaluates the mitochondrial function and acrosome reaction on thawed-frozen buffalo spermatozoa using flow cytometry.

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PRELIMINARY SEQUENCE ANALYSIS OF THE BUFFALO (Bubalus bubalis) CFTR GENE

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ABSTRACT

Eight regions corresponding to about 3 kb of the buffalo CFTR gene were amplified, using primers designed from the bovine CFTR gene, and sequenced to detect Single Nucleotide Polymorphisms (SNPs). In the whole, 12 polymorphic sites (9 transitions and 3 transversions) were observed, corresponding to an average density of 1 SNP every 250 bp. Out of the twelve polymorphic sites, three were in intron 8, one in exon 10, three in intron 10, three in exon 13, one in intron 13 and one in exon 19. Two SNPs in exon 13 are predicted to change the amino acid sequence (R to W and P to L, respectively). This initial SNP discovery phase will be further extended to include other CFTR target regions and to genotype larger samples in order to obtain reliable allele frequency estimates and to infer CFTR haplotypes that, together with the functional characterization of the sperm CFTR protein, will allow to evaluate the role of CFTR in sperm physiology.

INTRODUCTION

A number of molecular, biochemical, and physiological changes occur in sperm, during the incubation in the female reproductive tract *in vivo* or in defined media *in vitro*, leading to the acquisition of the fertilizing capacity. Main changes concern reorganization of the plasma membrane and increases in membrane fluidity, production of reactive oxygen species (ROS), increase in cAMP concentrations and protein tyrosine phosphorylation, changes in swimming patterns and chemotactic motility (hyperactivation), increase in intracellular pH and sperm plasma membrane hyperpolarization due to changes in the intracellular concentration of different ions, like Ca^{++} and $HCO3^-$. Several studies have demonstrated that capacitation is an $HCO3^-$ dependent process *(Lee and Storey, 1986; Neill*

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and Olds-Clarke, 1987; Boatman and Robbins, 1991; Shi and Roldan, 1995) and elevation of intracellular pH and hyperpolarization of the sperm plasma membrane observed during capacitation have been shown to depend on extra-cellular $HCO3^-$. The regulatory role of $HCO3^-$ in sperm capacitation has been attributed to its direct activation of a $HCO3^-$ sensitive soluble form of adenylyl cyclase *(Chen et al., 2000)* that in turns activates cAMP production and various downstream cellular events, such as protein tyrosine phosphorylation *(Visconti et al., 2002)*. Mechanisms of cellular $HCO3^-$ transport include CFTR (Cystic Fibrosis Transmembrane Conductance Regulator), a glycosylated protein that functions as a cAMP-regulated anion channel, known to conduct both Cl^- and $HCO3^-$ *(Ballard et al., 1999)* and to regulate several transporters and proteins. Recent evidences support the hypothesis that CFTR may play a fundamental role in mammalian sperm physiology and acquisition of the fertilizing ability. Xu et al. *(2007)* showed a significant decrease of fertilizing ability in heterozygous mutant mice carrying a CFTR mutation compared to homozygous wild-type mice. *In vitro* CFTR inhibition has been shown to significantly reduce mouse sperm capacitation *(Hernández-González et al., 2007; Xu et al., 2007)*, together with increase in intracellular pH *(Xu et al., 2007)*, and membrane hyperpolarization *(Hernández-González et al., 2007; Xu et al., 2007)* by blocking the influx of chloride and bicarbonate ions into the cell. Our ongoing research aims to define the role of the CFTR membrane protein in the acquisition of sperm fertilizing ability in buffalo. Here we present the preliminary results of the molecular characterization of the buffalo CFTR at the genomic level.

Fig.1. Alignment among the *Bubalus bubalis* CFTR exon 13(*) sequence and some publicly available mammalian and avian CFTR exon 13 sequences.

```
Bubalus_Bubalis           CTGAATTACAAAATCAGCGGCCCGACTTCAGCTCGAAGCTCATGGGATGTGACACTTTCGACCAGTTTACTCCAGAACGAAGAAATTCAATCATAACT
Bos_Taurus                CTGAATTACAAAATCAGCGGCCCGACTTCAGCTCGAAGCTCATGGGATGTGACACTTTCGACCAGTTTACTGCAGAACGAAGAAATTCAATCATAACT
Ovis_aries                CTGAATTACAAAATCAGCGGCCTGACTTCAGCTCGAAGCTCATGGGATGTGACACTTTCGACCAGTTTACTGCAGAACGAAGAAACTCAATCATAACC
Sus_scrofa                CTGAATTACAAAGTCAGCGGCCCGACTTCAGTTCAAAGCTTATGGGATATGATACTTTTGACCAATTTACTGCAGAACGGAGAAATTCAATCATAACT
Equus_caballus            CTGAATTGCAAAATCTACGGCCTGACTTCAGCTCAAAGCTCATGGGATATGATTCTTTCGACCAGTTTAGCGCAGAAAGAAGAAATTCAATCCTAACT
Homo_sapiens              CAGAACTCCAAAATCTACAGCCAGACTTTAGCTCAAAACTCATGGGATGTGATTCTTTCGACCAATTTAGTGCAGAAAGAAGAAATTCAATCCTAACT
Canis_lupus_familiaris    CTGAATTACAAAGTCTCCGGCCCGACTTCAGCTCGAAGCTCATGGGCTATGATTCTTTTGACCAGTTCAGTCCAGAAAGAAGAAACTCCATCATCACT
Felis_catus               CTGAATTACAAAATCTACGGCCTGACTTCAGCTCCAAGCTCATGGGATATGAGTCTTTTGACCAATTTAGTGCAGAAAGAAGAAATTCCATCATAACT
Mus_musculus              CTGAGCTACAAAGTCTACGTCCAGACTTCAGTTCGAAACTCATGGGGTATGATACTTTTGACCAGTTTACTGAGGAAAGAAGAAGTTCAATTCTAACT
Rattus_norvegicus         CTGAACTACAAAGTCTACGTCCAGACTTCAGTTCCAAACTCATGGGGTATGATACTTTTGACCAGTTTACTGAGGAAAGAAGGAGTTCAATTCTAACT
Gallus_gallus             CTGAACTTCAGGGTCAAAGGCCAGACTTCAGCTCAGAGCTGATGGGATTTGATTCTTTTGATCAATTCAGTGCAGAGAGAAGAAATTCAATTATAACT
                          * **  * **   **  †  ** ***** ** **  * ** ***** * ***  **** ** ** ** *     **  * ** *  ** **  * **

Bubalus_Bubalis           GACTTTACGGCGTTTCTCATTAGAAGGAGATACTTCTGTGTCCTGGAATGAAACGAAAAAGCCTTCTTTTAAACAGACTGGAGAGTTTGGTGAAAAAA
Bos_Taurus                GACTTTACGGCGTTTCTCATTAGAAGGAGATACTTCTGTGTCCTGGAATGAAACGAAAAAGCCTTCTTTTAAACAGACTGGAGAGTTTGGTGAAAAAA
Ovis_aries                GACTTTACGGCGTTTCTCATTAGAAGGAGATACTTCTGTGTCCTGGAATGAAACAAAAAAGCCTTCTTTTAAACAGACTGGAGAGTTTGGTGAAAAAA
Sus_scrofa                GACTTTACGGCGTTTCTCATTAGAAGGAGATGCCTCTGTGTCCTGGAACGAAACAAAAAAACAATCCTTTAAACAGACTGGAGAGTTTGGTGAAAAAA
Equus_caballus            GACTTTACGGCGTTTCTCATTAGAAGGAGATGCTACTGTCTCCTGGAACGAAACAAAAAAACAATCTTTTAAACAGACTGGAGAGTTTGGTGACAAAA
Homo_sapiens              GACCTTACACCGTTTCTCATTAGAAGGAGATGCTCCTGTCTCCTGGACAGAAACAAAAAAACAATCTTTTAAACAGACTGGAGAGTTTGGGGAAAAAA
Canis_lupus_familiaris    GACTTTACGGCGTTTCTCCTTAGAAGGAGATGCGGCCGTGCCCTGGAATGAAACAAAAAAGCAATCTTTTAAACAGACTGGAGAGTTTGGTGAAAAAA
Felis_catus               GACTTTACGGCGTTTCTCATTGGAAGGAGATGCGGCCGTCTCCTGGAATGAAACAAAAAAGCAGTCTTTTAAACAGACTGGAGAGCTTGGTGAAAAAA
Mus_musculus              GACCTTACGCAGGTTCTCAGTAGACG---ATTCCTCTGCCCCGTGGAGCAAACCCAAA---CAGTCGTTTAGACAGACTGGAGAGGTGGGAGAAAAAA
Rattus_norvegicus         GACCTTACGCAGGTTCTCAGTGGACG---ATGCCTCTACCACCTGGAACAAAGCCAAA---CAGTCATTTAGACAGACTGGAGAGTTTGGAGAAAAAA
Gallus_gallus             GACTCTGCGACGATTTTCCTTTGAAGGAGAAAGCATGGGATCACGAAATGAAATGAAGAAGCAGTCTTTTAAACAAACGTCAGATTTTAATGACAAAA
                          ***  * *   * ** **  * ** *   *           * * *   **   **    *  ** **** *** **   †**  *    ** ****

Bubalus_Bubalis           AAGAACTCCAT---TCTCAGTTCAATCAACTCTGTAAGGAAATTTTCAGTTGTACAAAAGACTTCATTACAAATGAATGGTATTGAAGGAGCTGCTGA
Bos_Taurus                AAGAACTCCAT---TCTCAGTTCAATCAACTCTATAAGGAAATTTTCAGTTGTACAAAAGACTTCATTACAAATGAATGGTATTGAAGGAGCTGCTGA
Ovis_aries                AAGAACTCCAT---TCTCAATTCTATCAACTCTATAAGGAAATTTTCAGTTGTACAAAAGACTTCATTACAAATGAATGGTATCGATGGAGCTTCTGA
Sus_scrofa                AAGAATTCCAT---TCTCAATTCAATCAACTCTATAAGGAAATTTTCAATTGTACAAAAGACTCCCTTACAAATGAATGGCTTTGAAGAAGATTCTGG
Equus_caballus            AAGAACTCTAT---TCTCAATCCAATCAACTCTATAAGGAAATTTTCAATTGTACAAAAGACTCCGTTACAAATGAATGGCATTGAAGAAGATTCTGA
Homo_sapiens              AAGAATTCTAT---TCTCAATCCAATCAACTCTATACGAAAATTTTCCATTGTGCAAAAGACTCCCTTACAAATGAATGGCATCGAAGAGGATTCTGA
Canis_lupus_familiaris    AAGAACTCTAT---TCTTAATCCAATCAACTCCATAAGAAAATTTTCAATTGTACAAAAGACCCCGTTACCCATGAATGGCATCGAAGGAGAGTCTGA
Felis_catus               AAGAACTCTAT---TCTTAATCCAATCAACTCTATAAGGAAATTTTCAATTGTTCAAAAGACTCCGTTACAAATGAATGGCATCGAAGGAGATCCTGA
Mus_musculus              AAGAACTCTAT---TCTAAATTCATTCAGCTCTGTAAGGAAAATTTCCATTGTGCAAAAGACTCCATTAT---------GTATCGATGGAGAGTCTGA
Rattus_norvegicus         AAGAACTCTAT---TCTAAGTTCATTCAGCTCTGTGAAGAAAATTTCCATTGTGCAAAAGACTCCATTGT---------CCATAGAAGGAGAATCTGA
Gallus_gallus             AAGAACTCCATAATTATTAACCCACTTAATGCAGGCAGGAAGTTATCGATAATGCAGAAGAATGGGACGCAGGTCAATGGACTGGAAGATGGCCACAT
                          ***** ** **   * * *   *  * *   *       **  * **  *  * ** ****                    * ** *  *

Bubalus_Bubalis           CGCCTTTAGAGAGAAGACTATCCTTAGTTCCCCATTCTGAACCTGGAGAG -475
Bos_Taurus                CGCCTTTAGAGAGAAGACTATCCTTAGTTCCCCATTCTGAACCTGGAGAG
Ovis_aries                AGCCTTTAGAGAGAAGACTATCCTTAGTTCCCCATTCTGAACCTGGAGAG
Sus_scrofa                AGCCTTTAGAGAGAAGGCTGTCCTTAGTTCCTGATTCTGAGCATGGGGAG
Equus_caballus            AGCCTTTGGAGAGAAGGCTGTCCTTAGTTCCGGATTCGGAGCAGGGAGAG
Homo_sapiens              AGCCTTTAGAGAGAAGGCTGTCCTTAGTACCAGATTCTGAGCAGGGAGAG
Canis_lupus_familiaris    TGCCAGTAGAGCGAAGGCTGTCCTTATTTCCCGACTGTGAGCAAGGAGAG
Felis_catus               AGCCTTTAGAGAGAAGGCTGTCCTTAGTTCCAGATGGCGAGCAAGGAGAG
Mus_musculus              ATCTCCAAGAAAAGAGACTGTCCCTAGTTCCGGATTCTGAACAGGGGGAG
Rattus_norvegicus         ACCTCCAAGAAAGGAGACTGTCCCTGGTTCCAGACTCTGAACATGGAGAG
Gallus_gallus             ACTCACCAGAAAGGAGGATCTCACTGGTACCTGACCTGGAACAGGGAGAT
                                **   **  * **  *  * *†  *    ** *  ** **
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(*) Only bases 126 to 425 are displayed. Variant sites between the buffalo and the bovine species are shaded. Buffalo SNPs are shaded and indicated by †.

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METHODS

Genomic DNA was extracted from frozen blood of Mediterranean Buffalo *(Bubalus bubalis)* animals sampled in the Province of Matera (Basilicata, Southern Italy) by using a commercial kit (FlexiGene DNA Kit, Qiagen). Sequence homology analysis using publicly available CFTR sequences revealed that the gene is highly conserved among different mammalian and avian species *(Fig. 1)*. Thus we designed PCR primers against ten different regions *(Tab.1)* evenly distributed along the bovine CFTR gene (Acc. N. NC_007302). PCR products were purified after agarose gel electrophoresis using QIAquick Gel Extraction Kit (Qiagen). Amplicons were sequenced using BigDye terminator sequencing chemistry. A sequence alignment analysis was carried out to identify nucleotide variants using the software Geneious Basic 3.7.0.

RESULTS

Alignment analysis among the *Bos taurus* CFTR and some mammalian and avian CFTR sequences available via NCBI revealed that the gene is highly conserved among different mammalian and avian species *(Fig.1)*. We, therefore, designed PCR primers against ten target regions, altogether accounting for about 4 kb of the bovine CFTR gene, to amplify the corresponding fragments from the buffalo genomic DNA. Two primers pairs failed to generate any specific amplified product (partial intron 14 to exon 15; exon 17b). Sequence homology analysis between the closely related buffalo and bovine species highlighted a high level of similarity (0,99% homology along all the sequences, 0,99% homology within the exonic regions and 0,93% homology within the intronic regions).
On the contrary, SNP analysis within the *Bubalus bubalis* species *(Tab. 2)* showed a considerable number of nucleotide substitutions, corresponding to an average density of 1 SNP every 250 bp. Out of 12 polymorphic sites, 9 were transitions and 3 were transversions, of them, 7 were intronic (A252C, C270T and A376G, in intron 8; A37G, C263T and A267G, in intron 10; A394G, in intron 13) and 5 were exonic (A111C, in exon 10; C143T, A307C and C453T, in the exon 13; A93G, in exon 19). SNPs C143T and C453T in exon 13 are predicted to change the amino acid sequence (R143W and P453L, respectively).

CONCLUSIONS

The CFTR gene is highly conserved also across distant species, but seems to be quite variable within the buffalo species. This initial SNP discovery phase will be further extended to

Table 1. CFTR genomic targets and list of the PCR primer pairs designed for this study.

SNP Regions	Forward Primer Sequence (5' - 3')	Reverse Primer Sequence (5' - 3')	T_a	Amplicon
Exon 8 partial intron 8	tgg aga atg taa cag cct tctg	tat gca aag cac gtt gga ctg gaa	56°C	538
Exon 10	tga tta tgg gag aat tgg aac ctt cag agg	atc ttt aat ggt tcc agg cat gat cca gga	53°C	104
Partial intron 10 exon 11	gaa tgt cta gca tgg ttg ttg c	aag aaa ttc tcg ctc gct gac ctc	58°C	778
Exon 11	gac atc tcc aag ttt gca gag aa	aag aaa ttc tcg ctc gct gac ctc	58°C	88
Exon 13	ctg aat tac aaa atc agc ggc ccg act tca gct	att tcg gct aag ctt gcc tga gga	56°C	526
Exon 13 partial intron 13	cgtct gaa cct tat gac cgg ctc	tat atg cct tgg aga ggc tac c	58°C	629
Partial intron 14 exon 15	aag tga agt gtg tgt tgc tcc	cac tgt gat tag agt atg cac cag	53°C	680
Exon 17b	tgt tat taa ttg tga tcg gag ctg tgg tgg tcg	att ctc att tgg aac cag cgc ag	53°C	319
Exon 19	tgc gat ctg tga gcc gag tc	gac agt cat ttg gcc ccc tga gg	56°C	170
Exon 12	tca gat ctg tga tag agc agt ttc ctg gga agc t	tgg atc caa atg agc act ggg tt	53°C	164

T_a, annealing temperature used in this study.

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include other CFTR target regions and to genotype larger samples in order to obtain reliable allele frequency estimates and to infer CFTR haplotypes that, together with the functional characterization of the sperm CFTR protein, will allow to evaluate the role of CFTR in buffalo sperm physiology.

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Table 2. Polymorphic sites in the *Bubalus bubalis* CFTR gene

SNP Regions	SNP Variants	Position *
Intron 8	A/C	252
Intron 8	C/T	270
Intron 8	A/G	376
Exon 10	A/C	111
Intron 10	A/G	25661
Intron 10	C/T	25887
Intron 10	A/G	25891
Exon 13	C/T	143
Exon 13	A/C	307
Exon 13	C/T	453
Intron 13	A/G	394
Exon 19	A/G	93

* Positions are numbered starting from the corresponding 5' intron/exon end.

CHROMOSOMAL ABNORMALITIES AND INFERTILITY IN RIVER BUFFALOES (*Bubalus bubalis*, 2N=50)

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INTRODUCTION

As known, buffaloes raised in Italy belong to the Asiatic water buffalo (river type, 2n=50). Their population has increased considerably (2% per year in the world and 8% in Italy) *(Borghese and Manzi, 2005)*. Breeders are very sensitive to reproductive problems. Indeed, very often females reach the reproductive age but do not show any oestrus manifestation or do not become pregnant even when the bull is present. In the last few years we have investigated females with reproductive problems by both clinical and cytogenetic analyses and found that an high percentage of these females (20%) were carriers of sex chromosome abnormalities. In this study we report an update on all chromosome abnormalities found in this species in Naples and Caserta provinces (where most buffaloes in Italy are raised).

MATERIAL AND METHODS

Two male co-twins, 119 females with reproductive problems and 13 males addressed to reproduction (bulls) were investigated. All females were visited by veterinary practioners by both external examination (body conformation, shape and size of head and horns, type of withers and pelvis) and rectal palpation (to check the internal sex adducts). Peripheral blood sample cultures were performed to obtain chromosome preparations. Two cell cultures were set up for 72 h for all animals: normal cultures without addition of base analogue and cultures treated for late incorporation of both BrdU (15 μg/ml) and Hoechst 33258 (30 μg/ml) in synchronised cell cultures to obtain R-banding patterns. CBA- and RBA-banding patterns were obtained from normal and BrdU-treated cells, respectively, following the protocols reported in Iannuzzi *(2003)*. Slides from some specific cases were treated for FISH-technique by using bovine or caprine BAC-clones and the technique reported in Iannuzzi *(2003)*. Karyotypes were constructed according to the standard karyotype of river buffalo *(CSKBB, 1994)*.

RESULTS AND DISCUSSION

All males addressed to the reproduction showed normal karyotype by using high resolution chromosome banding techniques *(Figure 1)*. When examining females with reproductive problems, an appreciable number of animals were found carriers of sex chromosome abnormalities as follows: X monosomy (2 females); X trisomy (one female); sex reversal syndrome (2 females); free-martinism (18 females and 2 males). A total of 25 animals (20%) were found carriers of chromosome abnormalities. In addition, all female carriers were sterile for serious damages to internal sex adducts varying between atrophy of Mullerian ducts to complete lack of internal sex adducts (with closed vagina), as revealed by direct visualization after mating *(Figure 2)* or rectal palpation performed by veterinarian practioners. However, although most of females showed normal body conformation and external genitalia, some females showed some male traits

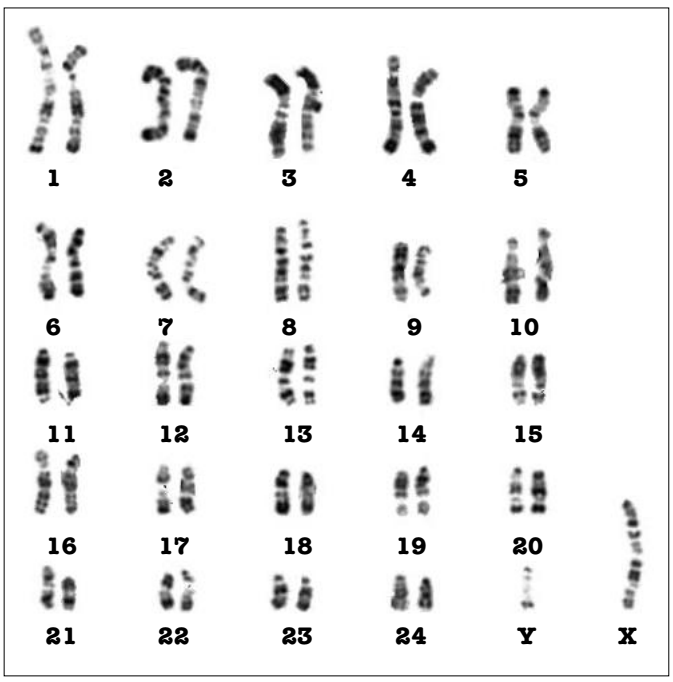

Figure 1. RBG-banded karyotype of river buffalo bull with normal karyotype (2n=50, XY)

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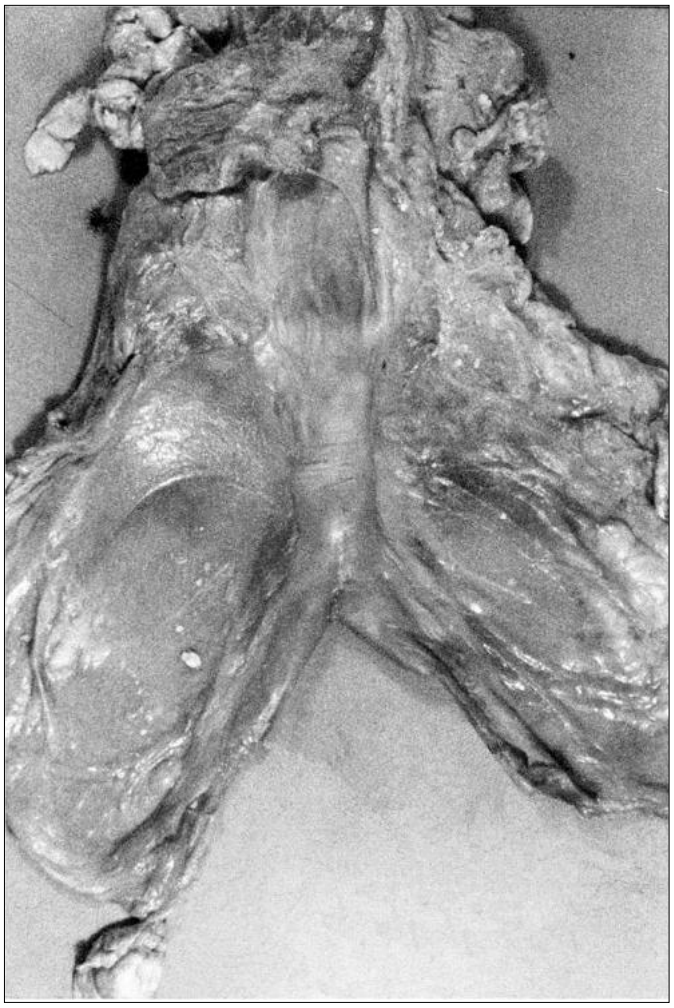

Figure 2. Atrophic internal sex adducts in a female affected by the mosaicism XX/XY (freematin).

such as large head and horns (circumference basis), prominent wither and tight pelvis *(Figure 3)*. This means that when the breeders and veterinary practioners note such small abnormal body conformation at the young age, a prompt cytogenetic analysis could reveal the presence or not of chromosome abnormalities so to eliminate the carriers from the farm avoiding to keep them for years with evident economical losses.

This study demonstrates the utility and the necessity to extend the cytogenetic analyses to all females with reproductive problems, especially when male traits were noted at the young age. This will genetically improve the river buffalo population and increase the adding value on the total buffalo production chain.

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Figure 3. Female river buffalo affected by X-trisomy (2n=51,XXX) with evident male traits (larger horns, prominent withers and tight pelvis).

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INFLUENCE OF GENETIC AND ENVIRONMENTAL FACTORS ON STILLBORN BUFFALO CALVES

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ABSTRACT

The subjects of the study were buffalo cows from the Bulgarian Murrah breed from the herd of the Agricultural Institute – Shumen. Out of the total calving number, 935 are heifers (first calving) and 2319 are adult buffaloes (with more than one calving). The total number of stillborn buffalo calves is 196. The cases of calving after the 270th day of the pregnancy with stillborn foetus have been included into the number of stillborn buffalo calves. The information on the stillborn buffalo calves has been taken from the herd's pedigree books and the veterinary register. Dispersion analysis of non-orthogonal complex for quality traits has been used for testing the influence of different factors on the frequency of stillborn buffalo calves *(Eftimov et al., 1972)*. The sources of variance are: genealogical line (i=1…6), age of buffalo cows (i=1…2) and season of calving (i=1…4). The arithmetic means (p) for the whole complex according to the levels of factors have been determined from the number of stillborn buffalo calves compared with the number of individuals in the variance sources. It has been established that thegenealogical line and season of calving have a certain influence on the stillborn buffalo calves and the age of buffaloes does not influence the frecuency of stillborn buffalo calves.

Keywords: buffalo cows, heifers, genealogical line, stillborn buffalo calves

INTRODUCTION

The effective production of buffalo farms is a result of a system of factors influencing the herds' reproduction and productivity. Studies show that the economic andgenetic losses most often are due to reproductive disturbances *(Tiwana and Bhalaru 1984;Bhalaru and Tiwana,1983; Zicarelli, 2000; Peeva, 2000; , 2007)*. According to Ilieva and Peeva *(2007)* one of the frequently met reproductive disturbances is stillborn buffalo calves, which are 5.6% of the total calving number and 42.3% of the total reproductive disturbances. The authors establish that 19% of the buffalo cows which have borne stillborn calves are later culled. The percentage of stillborn buffalo calves in other studies is in the range from 4.04 to 4.81 % of the total calving number *(Bhalaru and Tiwana, 1984, Prasad and Prasad, 1998)*. Bhalaru and Tiwana *(1983)* report about higher percentage of abortions and stillborn buffalo calves in heifers (15.9) than in buffalo cows (3.8). Ilieva and Peeva *(2007)* report about higher percentage of stillborn buffalo calves in buffalo cows (4.59) than in heifers (1.8). According to Tomar *(1984)* and Murudeppa *(1998)* the age of buffalo cows does not substantially influence the cases of stillborn buffalo calves.
The objective of this study is to establish the influence of the genealogical line, age of buffalo cows and season of calving on the frequency of stillborn buffalo calves.

MATERIAL AND METHODS

Subjects of the study were buffalo cows from the Bulgarian Murrah breed from the herd of the Agricultural Institute – Shumen. Out of the total calving number, 935 are heifers (first calving) and 2319 are buffalo cows (with more than one calving). The total number of stillborn buffalo calves is 196. The cases of calving after the 270th day of the pregnancy with stillborn foetus have been included into the number of stillborn buffalo calves. The information on the stillborn buffalo calves has been taken from the herd's pedigree books and the veterinary register. Dispersion analysis of non-orthogonal complex for quality traits has been used for testing the influence of different factors on the frequency of stillborn buffalo calves *(Eftimov et al., 1972)*. The sources of variance are: genealogical line (i=1…6), age of buffalo cows (i=1…2) and season of calving (i=1…4). The arithmetic means (p) for the whole complex and according to the levels of factors have been determined from the number of stillborn calves compared with the number of individuals in the variance sources.

RESULTS AND DISCUSSION

The results of the influence of the genealogical

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Table 1. **Variance analysis.**

Sources of variance	df	Sx,y	S2x,y	S (x,y)	F-test
Genealogical line	6	1,036	0,173	0,287	3,059**
Age of buffalo cows	1	0,011	0,011	0,048	0,191n.s.
Season of calving	3	1.282	0.427	0.362	7.602***

Table 2. **Influence of the factors on the stillborn buffalo calves.**

Sources of variance	Total number of calvings	Stillborn buffalo calves				
		n.	–	% compared:		
				Calvings for sources of variance	Total number of calvings	Total number of stillborn calves
Genealogical line						
Bull 1	339	13	0.038	3.84	0.40	6.60
Bull 12	1466	114	0.078	7.78	3.50	58.20
Bull 10	138	4	0.029	2.89	0.12	2.00
Bull 635	165	12	0.073	7.27	0.39	6.10
Bull 636	169	7	0.041	4.14	0,22	3,70
Bull 637	74	5	0.068	6.76	0,15	2,60
Others	903	41	0.045	4.54	1.26	20.80
Age of the buffaloes						
Heifers	935	58	0.062	6.20	1.81	30
Adult	2319	137	0.059	5.91	4.21	70
Season of calving						
Winter	579	59	0.102	10.19	1.81	30.10
Spring	867	49	0.056	5.65	1.51	25.00
Summer	1093	50	0.046	4.60	1.54	25.50
Autumn	715	38	0.053	5.31	1.16	19.40
Total:	3254	196	0.060	6.02	6.02	100.00

line, age of buffalo cows and season of calving on the frequency of stillborn buffalo calves are given in *Table 1*. The variance analysis indicates that the season of calving (F=7.602***) has the strongest influence on the frequency of stillborn buffalo calves followed by the genealogical line (F=3.059**). On the grounds of the insignificant effect of the age of buffalo cows we consider that the number of stillborn buffalo calves in buffalo cows and in heifers is not substantially different. This fact indicates that the age does not influence that trait.

The cases of stillborn buffalo calves according to the levels of factors are given in *Table 2*.

The comparative analysis between the progeny of the representatives of each genealogical line versus the total number of stillborn buffalo calves shows that Mati's progeny takes the highest relative part - 58.20%. The relative part of stillborn buffalo calves for the progeny of bull 1 and bull 635 is 6.6% and 6.1% respectively. The cases of stillborn buffalo calves are the fewest in the daughters whose sires are representatives of the genealogical line of bull 10.

The analysis of the percentage of stillborn buffalo calves to the calving number inside the line gives a better notion about the sire's

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influence on the frequency of the reproductive disturbance.
Regardless of the fact that the progeny of bull 12 is most numerous, the relative part of stillborn calves to the calving number of the line is 7.78%. The relative part of stillborn calves in the daughters from line of bull 635 and bull 637 has close values 7.27 and 6.76% respectively. The smallest relative part of stillborn calves is observed in the progeny of the bull _10 followed by the progeny of bull _1, 2.89 and 3.84% respectively.
There is not a substantial difference in the number of stillborn buffalo calves between heifers and buffalo cows. It means that the animals' age does not influence this reproductive disturbance.
The results of the season of calving show that the percentage of stillborn buffalo calves in winter is highest (10.19), although the relative part of calving is lowest.
It is most likely due to feeding and climatic conditions in winter. Regardless of the higher calving number in summer, the percentage of stillborn buffalo calves is lower (4.6). The stillborn buffalo calves in spring and autumn are 5.65 and 5.31% respectively. Out of the total number of stillborn buffalo calves (196) the lowest relative part belongs to those born in autumn 19.4 % and the highest one belongs to those born in winter - 30%.

CONCLUSIONS

The genealogical line and season of calving certainly has an influence on the stillborn buffalo calves.
The age of buffalo cows does not influence the number of stillborn buffalo calves.

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ARTIFICIAL INSEMINATION AND EMBRYO TRANSFER IN BUFFALO

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ABSTRACT

Artificial insemination (AI) in buffalo has limited use worldwide due the difficulties in the estrus detection and in finding an adequate moment for this procedure. Therefore, an alternative to increase the number of buffalo that are inseminated is the use of protocols that allow the AI without the need of estrus detection, usually called fixed-time AI (FTAI).

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Follicular wave development can be controlled by treatments with GnRH or estradiol and progestogen/progesterone in combination. Treatment of buffalo with GnRH in combination with prostaglandin F2α ($PGF_{2\alpha}$) 7 d later and a second GnRH 48 h after $PGF_{2\alpha}$ (known as Ovsynch) has resulted in acceptable pregnancy rates after FTAI in cycling buffalo during the breeding season. FTAI protocols using progestin devices, estradiol and eCG have resulted in synchronous onset of a new follicular wave, synchronous ovulation and consistent pregnancy rates in anestrous buffalo during the off breeding season. The combination of these protocols permits the use of AI throughout the year, obtaining conception and calving even in anestrus buffalo during the off breeding season. Superstimulation of follicular growth in buffaloes showed good response, however the embryo recovery is only 20 to 30% of ovulations. Results provide strong evidence that low embryo recoveries in buffalo may be explained by a failure of oocyte to entry the oviduct after superovulation. The calving of buffaloes produced by *in vitro* embryo showed that it is possible to obtain an IVF protocol to this specie. Although, more studies must be performed to increase the ET and IVF results allowing the commercial use in buffaloes.

Keywords: Artificial insemination, Ovulation synchronization, Progesterone, Buffalo

INTRODUCTION

Associated to the enhancement in reproductive efficiency, techniques used to achieve genetic improvement make possible to obtain herds with better productive characteristics, such as growth rate, carcass quality, milk yield, food conversion and precocity, among others. Thus, multiplication of superior animals by using reproductive biotechniques [artificial insemination (AI) and embryo transfer ET)] can provide greater economic return. Moreover, increased reproductive rates associated to genetic improvement must be the main objective of farmers to improve buffalo productivity and farms income. Therefore, the objective of this review is to present the results of our research trials, in order to provide information that could be utilized to increase the use of AI and ET in commercial buffalo herds.

ARTIFICIAL INSEMINATION

One of the major factors that impair reproductive development in artificially inseminated herds is the failure in estrus detection. The correct management of estrus detection requires continuous observation of the herd and qualified, responsible and knowledgeable labour. In herds in which estrus detection is inefficient, there is a decrease in the reproductive performance and a consequent increase in the breeding period and in the calving interval, what causes serious economic losses for the breeder.
Therefore, the use of management schemes that do not require the identification of estrus contribute for the increase in the use of AI in buffalo herds, mainly because it is easy to perform. The objectives of these schemes are to synchronize the luteal phase, the follicular growth and the ovulation allowing the AI in all animals of the farm, even those that are not showing estrus or cyclicity. The use of these protocols has been collaborated with the widespread of the artificial insemination in buffalo herds, and enables genetic improvement, increasing milk and meat yield.
To archive these targets, many researches were conducted in Brazil in order to evaluate the synchronization of ovulation protocol efficiency for fixed-time artificial insemination (FTAI) in buffaloes *(Baruselli, 1999; Baruselli et al., 1999b; Baruselli et al., 2000)*. However, the reproductive seasonality observed in buffaloes (Zicarelli, 1994) can interfere in the efficiency of the synchronization of ovulation in protocols for FTAI. At the period of seasonal anestrus buffaloes present absence of estrous behavior and a lack of ovulation and progesterone secretion by the ovary. Thus, at this period ovarian follicular turnover occurs. However, our data showed that estrus and/or ovulation may be induced in anestrous buffalo by hormonal treatment. Hence, it is important to know that different protocols may be utilized in buffalo according to the breeding season and the cyclicity status of the herds. Nowadays, the FTAI protocols are already established for buffaloes and have been used in several farms in Brazil throughout the year.

SYNCHRONIZATION OF OVULATION FOR FTAI DURING THE BREEDING SEASON

Our research team developed an experiment in order to evaluate the synchronization of ovulation protocol efficiency, when GnRH and prostaglandin ($PGF_{2\alpha}$) were used for FTAI in buffalo *(Baruselli et al., 1999a, b)*. Females received a treatment (known as Ovsynch). The objective of this trial was to determine the conception rate (CR) to FTAI in buffalo (n=1,053) treated for synchronization of

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ovulation with the GnRH/ PGF2a/ GnRH protocol (Ovsynch) during the breeding and off breeding seasons *(Baruselli et al., 1999b)*. The effects of body condition score (BCS; 1 to 5 scale), parity, postpartum period, and the breeding season on CR were analysed.
CR was influenced by BCS. Females with BCS ≤ 3.0 presented 31.4% of CR (n=223), while in females with higher BCS were observed increase of CR [3.5 = 52.9% (n=546) and. ≥ 4.0 = 57.1% (n=198)]. Other trials also verified strong effect of BCS in CR in artificially inseminated buffalo after estrus detection *(Bhalaru et al., 1987, Baruselli et al., 2001)*. Lower BCS has been correlated with postpartum anestrus. Rasby et al. *(1992)* reported that nutrition restriction has a negative influence on LH release. Animals in anestrus showed decrease in diameter of the dominant follicle and in ovulation rate to the GnRH treatment. The results suggest that buffaloes may have to present BCS ≥ 3.5 for a satisfactory response to the treatment with GnRH and prostaglandins for FTAI.
CR was also affected by parity. Lower CR ($P<0.05$) was observed in primiparous compared to pluriparous cows [35.5% (49/138) vs. 51.0% (423/829)]. Probably, the decrease in the CR in primiparous could be explained by the increase in the number of buffaloes in postpartum anestrus Good nutritional management in primiparous could increase cyclicity and the CR after FTAI protocols. If the buffaloes are kept at the same nutritional management, specially presented in buffalo maintained in tropical pasture conditions, pluriparous cows should be preferentially synchronized, in order to improve the efficiency of this treatment.
CR was not affected by postpartum period. Buffaloes inseminated at < 60days post partum presented the same CR (50.9%; 170/334) than buffalo inseminated between 60 to 99 days (48.2%; 158/328) and > 100 days postpartum (47.2%; 144/305). To obtain 12 month intercalving interval, buffalos need to become pregnant 60 days after the calving. These results show that it is possible to have a good reproductive performance using early FTAI protocols (between 40 to 60 days after calving).
Breeding season influenced CR. Buffaloes treated during the breeding season (autumn and winter) presented higher CR than buffalo treated during the off breeding season [48.8% (472/967) vs. 6.9% (6/86)]. This demonstrated that even with exogenous hormonal stimulation using GnRH and prostaglandins, buffalo continue to present marked reproductive seasonality, which had also been demonstrated by several trials *(Zicarelli, 1994, Baruselli, 1994; Zicarelli, 1997)*. These results suggest that buffalo in seasonal anestrous do not respond to Ovsynch protocol.
These data demonstrate that the use of Ovsynch protocol for FTAI is feasible in buffalo. However, in order to obtain better results, the females should present good BCS, be preferentially pluriparous, and the procedure should be performed in cycling buffaloes during the breeding season (autumn and winter).

SYNCHRONIZATION OF OVULATION FOR FTAI DURING THE OFF BREEDING SEASON

As shown previously, the Ovsynch protocol present low conception rate after FTAI in buffaloes during the off breeding season. At this season buffalo often have a high incidence of anestrus, which extends the calving to conception interval and, consequently, negatively affects their reproductive performance. Previous studies carried out in postpartum anestrous cows have demonstrated that progesterone treatment stimulates an increase in LH pulse frequency during and following the treatment period *(Rhodes et al., 2002)*. Treatment of anestrous cows with progesterone resulted in greater follicular fluid and circulating concentrations of estradiol, increased pulsatile release of LH and increased numbers of receptors for LH in granulosa and theca cells in preovulatory follicles, compared with untreated animals *(Rhodes et al., 2003)*. Furthermore, a short period of elevated progesterone concentrations during anestrus period is important for the expression of estrus as well as subsequently normal luteal function *(McDougall et al., 1992)*. Thus, it is hypothesized that exposure of anestrous buffalo to progesterone may stimulate development and maturation of a dominant follicle by enhancing release of LH and stimulating development of LH receptors and secretion of estradiol, and then promoting the ovulation.
Studies have been performed by our group to evaluate progesterone treatments in conjunction with estradiol in various ovulation synchronization protocols in buffalo. Our group evaluated two protocols for synchronization of ovulation for buffalo FTAI (Ovsynch vs. P4+EB+eCG) during the off breeding season (Baruselli et al., 2002). In this trial, the Ovsynch Group (n=39), was submitted to the traditional GnRH/ $PGF_{2\alpha}$/ GnRH treatment and inseminated 16 hours after 2nd GnRH. The

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buffaloes in the P4+EB+eCG Group (n=96) received a intravaginal P_4 device (CIDR-B®) plus 2.0 mg EB (Day 0, PM). The CIDR-B® was removed and a luteolytic dose of $PGF_{2\alpha}$ and 500 IU of eCG were administered on Day 9 (PM). After two days (Day 11, PM), buffaloes received 1500 IU of hCG i.m. Females were FTAI 14h after the hCG treatment (D12, PM). The CR of FTAI buffaloes were higher in animals treated with P4+EB+eCG (53.5%) compared to the animals treated with GnRH/ $PGF_{2\alpha}$/ GnRH (28.2%; P<0.01). CR in buffaloes treated with P4+EB+eCG was considered satisfactory, since the protocol was performed during the off breeding season.

A significant benefit of using this protocol is that buffalo milk yield can be homogeneously distributed throughout the year, without concentrating the calving and milk yield in determined periods of the year. However, the costs of this protocol are higher than the Ovsynch protocol, used during the breeding season (autumn and winter).

Despite the positive effect of progesterone on follicular growth and ovulation, there are reports that the ovulation rates at the end of the treatment with progestin may compromise according to anestrus depth. Thus, the stimulation with a treatment using gonadotrophin can collaborate to improve the responses to synchronization of ovulation protocols.

The placental hormone eCG (Equine chorionic gonadotrophin) has both FSH- and LH-like activity and it parenteral administration stimulates follicular growth and ovulation in cattle *(Soumano and Price, 1997)*. The insertion of subcutaneous norgestomet ear implants or intravaginal progesterone devices, combined with the application of eCG at the time of device removal has been extensively used in *Bos indicus* herds with high incidence of postpartum anestrus *(reviewed in Baruselli et al. 2004)*. The use of 400 IU of eCG at the time of progestin device removal resulted in increased ovulation and pregnancy rates in cows without a CL at the time of insertion of the progestin device *(Baruselli et al. 2003)*. It was also verified that the eCG treatment increased plasma progesterone concentrations and pregnancy rates in suckled cows treated during postpartum anestrus *(Baruselli et al. 2004)*. Therefore, eCG treatment may be an important tool for increasing pregnancy rates at FTAI protocols in anestrous buffalo.

We realized an experiment to study the efficiency of the eCG treatment at the time of device removal during the off breeding season *(Porto Filho, 2004)*. Buffaloes received an intravaginal progesterone device associated with 2mg of EB on D0. On D9 the device was removed and PGF was administered. In the control group no eCG was used and in eCG group 500IU of eCG was administered at the time of device removal. The follicular response was monitored by ultrasonography. The ovulation rate was 44.4% in control group and 70.0% in eCG group. This result shows an increase in the follicular response after the treatments using progesterone/ progestogen associated with estradiol. It is in agreement with the benefits of the treatment with eCG also in buffalo synchronized during the off breeding season.

In another study was evaluated the effect of intravaginal progesterone device (DIB) and an auricular norgestomet implant (Crestar plus 2mg of EB at the time of insertion) on follicular response and on conception rate in buffaloes during the off breeding season *(Carvalho et al., 2007)*. The buffaloes treated with DIB and Crestar presented similar diameter of the dominant follicle on D9 (1.0 ± 0.1 cm vs. 1.0 ± 0.1 cm), diameter of ovulatory follicle (1.3 ± 0.1 cm vs. 1.3 ± 0.0 cm), interval between GnRH and ovulation (29.1 ± 3.1 h vs. 29.0 ± 4.4 h), ovulation rate [77.8% (7/9) vs. 92.3% (12/13)], and conception rate [43.7% (14/32) vs. 50.0% (28/56)]. Both treatments provided satisfactory follicular response and conception rate in buffalo during the off breeding season. However, the response to any treatment in off breeding season buffalo appears to be dependent on some factors that influence 'depth' of anestrus (i.e., seasonality – distance of buffalo herd from equatorial line –, body condition, age, and interval from calving).

SUPERSTIMULATION AND EMBRYO TRANSFER

Bovine embryo transfer has been applied widely around the world. This technology increases the number of offspring obtained from donors with high genetic value and is used to disseminate desirable genetics around the world. However, buffalo embryo transfer present low efficiency compare to bovine, difficulting the use of this important technique by buffalo farmers.

In our trials buffalo present acceptable follicular response during superovulation (10 to 15 follicles ≥ 8 mm), moderate ovulation rate (~ 60%) and CL yield (5-10) but, in contrast, a low embryo recovery rate is observed (20 to 30% of ovulations; *Baruselli, 1997a*). We postulate,

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therefore, that a high proportion of oocyte fails to enter the oviduct after superstimulation.
It was also observed that buffalo presented, in average, ovulation rates of 62.8%, which is similar to the one found for bovines *(Desaulniers, et al., 1995; Shaw et al., 1995; Stock et al., 1996)*. This result suggests that the low efficiency of MOET is probably not related to follicular response and to ovulation during superstimulation treatment.
Due to the low embryo recovery rate found for buffalo, our group performed a trial in order to evaluate superstimultion treatment by *in vivo* ultra-sound examination and by visual inspection of the ovaries after slaughter of the animals *(Baruselli et al., 2000)*. The objective in this study was to examine whether low embryo recovery rate in superstimulated buffalo is due to a failure of either follicle growth, ovulation or entry of oocytes into the oviduct. Ovarian follicular growth was recorded using rectal ultrasonography every 12 h from oestrus to ovulation. Animals were slaughtered 3.5 to 5.5 d after estrus, and the oviduct and uteri were flushed to recover embryos/oocytes
Superstimulation resulted in 17.2 ± 5.7 follicles $\geq$ 8mm; 9.2 ± 3.8 (53.5%) ovulations; and 3.2 ± 2.6 embryos + oocytes recovered. The embryo recovered represented 34.8% of ovulations. The percentage of oocyte recovery relative to CL was the same for buffalo slaughter on day 3.5, 4.5 or 5.5 after estrus. Sectioning (50mm) of corpora lutea in one buffalo did not show retained oocytes, suggesting ovulations. It would appear that a high proportion of oocyte fails to enter the oviduct after superstimulation of follicular growth in buffalo. The results provide strong evidence that low embryo recoveries reported previously in buffalo may be explained this fact.
Buffalo have relatively low plasma concentrations of 17β-estradiol during normal oestrous cycles (~7pg/ml) when compared to cattle *(Batra et al., 1983; Porto Filho, 2000)*. The circulating concentrations of estradiol are relatively high (~27pg/ml) during superstimulation in buffalo *(Baruselli et al., 2000a)* and may adversely effect the functioning of the infundibulum and passage of ova into the oviducts. Follicles in superstimulated buffalo that did not ovulate in response to the endogenous LH surge continued to secrete relatively large amounts of estradiol *(Schallenberger et al., 1990)*. Misra et al. *(1998)*, demonstrated that high oestrogen concentrations in superovulatory buffalo may affect embryo recovery rates.
A GnRH agonist-LH protocol for superstimulation was developed in cattle *(D'Occhio et al., 1999)*. In this protocol, the endogenous pre-ovulatory surge release of LH is blocked by treatment with a GnRH agonist implant and ovulation subsequent to follicular superstimulation is induced by injection of exogenous LH. Bovines treated with the GnRH agonist-LH protocol tended to have lower plasma concentrations of estradiol compared to Bovines treated with a conventional superstimulation protocol *(D'Occhio et al., 1999)*. The potential to use a GnRH agonist bioimplant and injection of exogenous LH to control the time of ovulation in a MOET protocol was examined in buffalo *(Carvalho et al., 2002)*. It has been shown that the GnRH agonist-LH protocol can be used to improve the efficiency of MOET in buffalo, but, the number of embryos collect is still inadequate.
Another experiment was done to block the plasma estradiol concentration in superstimulated buffalo *(Baruselli et al., 2002a)*. The objective of this experiment was to examine if the presence of exogenous progesterone during the expected estradiol surge could decrease plasma estradiol concentration and increase the embryo recovery rate. The results obtained shown that superstimulated buffalo in presence of CIDR-B®, during the period between $PGF_{2\alpha}$ and LH injections had lower ovulation rate and lower estradiol levels compared with the control group. Although the estradiol concentration was decreased, the embryo recovery rate remained low.
It known that the recombinant bovine somatrotropin (rBST) increase the antral follicular population and improve the oocytes quality for direct or indirect effect *(Gong et al., 1996)*. The treatment with rBST active the cumulus cell expansion that can contribute to interaction oocyte/tuba and then to increase the embryo recovery rate in superstimulated buffalo. Recently, Baruselli et al. *(2003a)* verified the efficiency of the rBST in a MOET protocol. It was used 16 buffaloes that were randomly allocated in 2 Groups (Control, n=8; rBST, n=8). Buffaloes were slaughtered on the flushing day and the oviduct and uterus were flushed. The numbers of follicles $\geq$ 3mm at the beginning of FSH treatment (Control=12.4 ± 3.6 vs rBST=15.6 ± 3.7) and $\geq$ 8mm at LH treatment (Control=12.7 ± 7.0 vs rBST=18.6 ± 8.4) were similar (P>0.05) between groups. Buffaloes treated with rBST increased (P<0.05) the ovulation rate (Control=38.2% vs rBST=55.0%) and embryo recovery rate (Control=33.3% vs rBST=50.0%), and numerically the number of CL (Control=4.9 ±

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3.2 vs rBST=10.2 ± 9.4) and embryos recovered (Control=1.6 ± 1.7 vs rBST=5.1 ± 6.8).
However, other studies performed by our research group did not confirm the increase of response when rBST was used in superstimulated buffaloes *(Carvalho et al. 2007a)*.
In spite of the fact that there are problems related to the low efficiency in the collection of embryo structures, there are reports on the birth of buffalo embryo using the embryo transfer technique in Brazil *(Baruselli et al., 1994)*. There are also reports on the birth of calves from frozen embryos *(Baruselli, 2000)*, what demonstrates the feasibility of the technique, provided that the rate of embryo recovery is improved.

IN VITRO PRODUCTION OF BUFFALO EMBRYOS

Buffalo respond less to multiple ovulation and embryo transfer procedures in comparison to cattle. Given these consideration, utilizing OPU technology in conjunction with in vitro embryo production (IVP) presents the possibility of enhancing genetic progression through the female lineage *(Galli et al., 2001; Gasparrini, 2002; Neglia et al., 2003)*. Although there has been interest in applying OPU and IVP techniques in buffalo, certain factors have limited the commercial use of OPU-IVP in buffalo. These include the lesser number of primordial follicles present in the ovaries *(Danell et al., 1987, Baruselli et al., 1997)*, lesser number of oocytes recovered per OPU session *(Gasparrini, 2002; Gasparrini et al., 2006)*, greater incidence of atretic follicles *(Le Van Ty et al., 1989)* and oocytes, and lesser cleavage rates *(Gasparrini, 2002; Gasparrini et al., 2006)*.
The OPU has been applied in buffaloes *(Boni et al., 1997)*, however, the results showed low efficiency *(Gasparrini, 2002)*. The *in vitro* embryo production depends of some steps. The first step is the oocytes recovery from ovaries of slaughter houses or live animals guided by ultrasound of follicles. The next step is the *in vitro* maturation of oocytes. This step is followed by *in vitro* fecundation and then the cultivation until morulae and blastocyst. The produced embryos could be transferred or cryopreserved and stocked *(Gasparrini, 2002)*. The quality of recovered embryos is determinant for embryo production *in vitro* and then availability of commercial OPU-FIV technique in buffaloes *(Ohashi et al., 2003)*.
Studies performed using rBST showed an increased number of little antral follicles and an improvement of oocytes quality caused by a direct and/or indirect effect of insulin-like growth factor type I *(IGF-I –Lucy, 2000)*. The treatment with rBST stimulates the expansion of *cummulus* cells *(Izadyar et al., 1998)*, improving the oocytes quality. bST treatment successfully increased the follicular population, demonstrating its potential for enhancing the efficiency of OPU programs in buffalo *(Sá Filho et al., 2005)*. Ferraz et al. *(2005)* used OPU and *in vitro* production of buffalo embryo allocated in two groups (rBST Group and Control Group). It was verified that rBST treated animals had higher number of collected follicles than Control Group (12.3 vs. 15.7; $P<0.05$). However, the quality of oocyte was higher in Control Group than in rBST Group (1.8 vs 0.9; $P<0.05$). The positive effect of rBST could be hidden by the dosage used in this experiment. Lower dosage must be studied to verify if it is possible to obtain positive effects.
These data showed that new researches are needed to the development of this technique and improve the results of OPU-FIV in buffaloes. The obtaining of first pregnancies and calving in buffalo produced *in vitro* in the world demonstrated that it could be possible to obtain an efficient OPU-FIV protocol to buffalo.

CONCLUSION

Currently, the world's economic situation requires efficient management practices to increase the profitability of buffalo operations. Optimal reproductive efficiency is crucial to increase net returns. The use of animal breeding technologies, as the artificial insemination, has become of great importance, particularly to introduce high genetics into buffalo herds. However, the reproductive seasonality and the time and effort required to perform estrus detection have limited the widespread application and success of this technology. The incorporation of techniques designed to control follicular wave dynamics and ovulation in recent years has reduced problems associated with estrus detection. Follicular wave development can be controlled by treatments with GnRH in combination with $PGF_{2\alpha}$ 7 d later and a second GnRH 48 h after PGF (known as Ovsynch). This protocol has resulted in acceptable pregnancy rates after FTAI in cycling buffalo during the breading season. Furthermore, treatments with progesterone/ progestogen, estradiol, eCG and GnRH/ hCG have provided possibilities for the

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application of FTAI in off breeding season, promoting earlier resumption of ovulation and cyclicity in buffalo in anestrus. Nowadays, the fixed time artificial insemination may be utilized throughout the year, in order to schedule efficiently the conception and the calving period. However, it is very important to recognize that the success of breeding programs will also depend on many management factors, such as the nutritional and health management, availability of qualified personnel, facilities and the objectives of the breeding program. Buffalo embryo transfer present low efficiency compare to bovine, making difficulty the use of this important technique by buffalo farmers. Other studies must be performed to verify if the increase of oocyte quality, the block of a–adrenergic receptors of oviduct and/or the decrease of estrogen/progesterone ratio could increase the recovery rate of embryo structures on buffaloes submitted to MOET programs. Buffaloes calves by OPU-FIV programs in the world showed that the technique could be available, however, more studies are needed to improve the efficiency to allow the commercial use in buffaloes.

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EMBRYONIC MORTALITY IN BUFFALO COWS

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ABSTRACT

Buffalo species show a tendency to a seasonal reproductive activity and it can be considered a "short day" species. Oestrus cycles occur throughout the year but the frequency of anoestrus and ovarian inactivity is higher and fertility is lower when daylight hours increase. Unfortunately, seasonal reproduction cause an increase of milk availability in autumn winter

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period while in Italy milk market demand increase in the spring summer period. To avoid market problem, the out of breeding season mating technique is widely applied in Italy and animals are forced to bred mainly in the spring-summer period. Its employment, however, cause longer intercalving periods, a consequent decline in herd fertility and higher culling rate.
Also in buffalo, embryo mortality is considered one of the major causes of herd fertility decrease, especially in animals mated when the daylight hours increase. Different trials showed that percentage of embryonic loss in animals mated by artificial insemination (AI) varied between 20% and 40% during seasons characterized by positive photoperiod whereas values of around 7% were recorded in Brazil during decreasing daylight hours.
Much of the loss of potential offspring in buffalo seems to occur later than in bovine, between 25 and 40 days from AI and in buffaloes naturally mated its percentage varied between 8.8% and 13.4% respectively at 28-45 days (embryonic mortality) and 46-90 days (foetal mortality) of pregnancy, regardless of conception period. In this trial no differences were found in EM incidence between different conception periods, while a significant high incidence of FM was found in December-March period compared to the April-July period. Embryonic mortality in buffalo species was not affected by age, parity or days in milk and infectious agents explained only about 2-8% of the cases. A reduced capacity to secrete progesterone (P4) seems to explain in part this embryonic mortality but other as yet unidentified factors could contribute between 40-50% to the embryonic losses. Several approaches have been used to increase P4concentration in blood in order to reduce the occurrence of embryonic mortality. Increased P4 plasma levels were achieved either by inducing increased endogenous secretion or by administering exogenous P4. The injection of a GnRH agonist on Day 5 after AI increased milk whey P4 concentrations in 97% of buffaloes subsequently pregnant on Day 40, compared to 68% in the non-pregnant buffaloes ($P<0.01$). It is however acknowledged that whilst P4 tended to increase on Day 10 in buffaloes treated with buserelin and hCG, its levels were significantly higher than control ones only on Day 15. These findings suggests that hCG and buserelin administration did not act by increasing P4 secretion from the existing corpus luteum but by inducing a new ovulation and the formation of an accessory corpus luteum. A greater ($P<0.05$) proportion of the buffaloes that ovulated (96.7%), compared to buffalo that did not ovulate (68.4%) recorded a gestational chamber on Day 40 after AI and were judged to be pregnant. Ovulation also increased milk whey P4 levels and reduced embryonic mortality in buffalo cows treated with 1500 I.U. of hCG or 12.6 µg of GnRH agonist on Day 25 after AI

Keywords: Embryonic mortality, Buffalo, Progesterone, GnRH, hCG.

Buffalo reproductive characteristics.
Buffalo species show a tendency to seasonal reproductive activity and, like sheep, it can be considered a “short day” specie. Oestrus cycles occur throughout the year but the frequency of anoestrus and ovarian inactivity is higher and fertility is lower when daylight hours increase. Buffalo bred in the autumn- winter period show an intercalving period of less than 400 days and a culling rate of less than 12% as observed in Italy, Brazil, Venezuela, and Argentina *(Zicarelli et al., 1993).*
Unfortunately, seasonal reproduction cause an increase of milk availability in autumn winter period while in Italy milk market demand increase in the spring summer period. To avoid market problem, the out of breeding season mating technique is widely applied in Italy. Its employment, however, cause longer intercalving periods, a consequent decline in herd fertility and higher culling rate *(Zicarelli, 1997; Campanile, 1997; Gasparrini B., 2002).* In buffalo, in fact, the resumption of ovarian activity is affected by the calving season: buffaloes that delivered during the spring period, showed an intercalving period on average longer by 30 or 70 days than those mated in the autumn-winter period, respectively if they are pluriparous or primiparous *(Zicarelli, 1994).* Usually, after 90 days of lactation during the spring period, 44% of pluriparous and 80% of primiparous are acyclic *(Zicarelli, 1994).*
Climatic variation also influence buffalo reproductive activity: temperatures lower than 8°C and daylight hours higher than 11 hours cause delayed ovulation probably due to a delay in the pituitary response to ovarian steroid secretion *(Zicarelli et al., 1988a).* Thermal excursions higher than 7°C - 9°C appreciably increase the incidence of double ovulations in both spontaneous and induced oestrus *(Zicarelli et al., 1988b).* Moreover, Sastry and Georgie *(1978)* found significant correlations between pregnancy rate and temperature, relative

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humidity or rainfall with lower temperature and increased rainfall improving conception rate.

Embryo development.

A suitable uterine environment is essential for embryo implantation and the maintenance of of uterine quiescence, nourishment and survival of the embryo/foetus gestation in cattle *(Ulberg et al., 1951; McDonald et al., 1952)* and buffalo (Campanile, 2006). Furthermore, P4 prepares the uterus for recognition of pregnancy *(Vincent and Inskeep, 1986; Vincent et al., 1986)*. In bovine first embryo cleavage occur at about 30 hours after insemination and the second at around 48 hours. During the following 4-5 days, subsequent blastomere divisions in the oviduct yield 4, 8, 16, 32 cells embryo, tight morula and, finally, blastocyst *(Senger P.L, 2003).* On day 6.5-7 embryo reaches the uterus and during the second week of gestation it starts to elongate and to send signals to the mother, in order to prevent luteolysis allowing adequate P4 circulating levels. Karaivanov et al. *(1987)* found that in superovulated buffalo oocytes could be recovered only from the oviducts between 74 and 100 h after ovulation, while at 102-108 h they could be found both in the oviducts and in the uterine horns. This findings suggests that embryo development could be faster in buffalo than in bovine, as confirmed by a trial carried out in vitro, during which tight morulae and blastocysts were observed already on day 5-6 *(Neglia, et al., 2001).*

Embryonic mortality.

Embryonic loss increase when physiological regulation of oviduct and uterine function is inadequate or when the mother is exposed to one or more stressors that can compromise embryonic survival *(Hansen, 2002)*. In cattle much of the loss of potential offspring is concentrated in the embryonic period, the first 42 d after breeding *(Inskeep E. K., 2004)* and up to 40% of total embryonic losses occur between days 8 and 17 of pregnancy *(Humblot P.,2001; Thatcher et al. 2001).* Vasconcelos et al. *(1997)* recorded that during the subsequent phases of pregnancy (after 42 days), potential offspring loss is a remote eventuality (around 10%).

In buffalo also embryo mortality is considered one of the major causes of herd fertility decrease, especially in animals mated when the daylight hours increase. Different trials showed that percentage of embryonic loss in animals mated by artificial insemination (AI) varied between 20% and 40% during seasons characterized by positive photoperiod *(Campanile et al., 2005; Campanile et al., 2007a; Campanile et al., 2007b)*, whereas values of around 7% were recorded in Brazil during decreasing daylight hours *(Baruselli et al., 1997)*. In spite of daylight variations, an embryonic mortality rate of 20% was also reported for buffaloes bred close to the equator *(Vale et al.,1989)*.

Embryo mortality in buffalo seems to occur later than in bovine, between 25 and 40 days from AI *(Campanile et al., 2005)*. In buffaloes naturally mated *(Vecchio et al., 2007)*, percentage of potential offspring loss was 8.8% and 13.4% respectively at 28-45 days (embryonic mortality EM) and 46-90 days (foetal mortality – FM) of pregnancy, regardless of conception period. In this trial no differences were found in EM incidence between different conception periods, while a significant high incidence of FM was found in December-March period compared to the April-July period. It was hypothesized that in the December March period there was a higher incidence of buffaloes that became pregnant in spite of a lower function of the corpus luteum due to a taking over anoestrus. In the subsequent months (April-July) an increased incidence of acyclic buffaloes was observed and, hence, only buffaloes that didn't care of the photoperiod were cyclic and became pregnant. In fact, the incidence of FM was similar to that observed in the decreasing daylight length period (August-November), the most favourable period for reproductive activity. These data are in accordance with Baruselli (personal communication), that found a pregnancy loss, after 30 days from AI, of 13.2% and 7.0% respectively in decreasing and increasing daylight length periods. In 1994, Zicarelli *(1994)* found an EM of 21.8% in buffaloes naturally mated without any influence of the breeding season (spring vs. summer), different between farms and correlated with the pregnancy –first oestrus period.

In another trial performed on 3000 conceptions *(Zicarelli, L., unpublished data)*, a higher incidence of EM was reported between 30 and 90 days of pregnancy in buffaloes that conceived during increasing daylight length. However, also in this case, farm, management and environment effects significantly affected EM incidence. Percentage of EM varied from 5% to 14%, but both values were lower than those previously recorded *(Zicarelli L., 1994).* The incidence of EM found in Italy was higher between 28-60 days of pregnancy and lower after 71 days while in cattle *(Silke et al., 2002)*

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no difference was found in EM from 28-87 days of gestation.
EM in buffalo was not affected by age, parity or days in milk and infectious agents explained only about 2-8% of the cases *(Campanile et al., 2005; Campanile et al., 2007a).* Campanile *et al. (2005)* found higher P4 plasma levels since day 10 after AI in pregnant buffaloes than in buffaloes which showed EM at 40 days of pregnancy whilst P4 in non-pregnant buffaloes was intermediate.
Buffalo diagnosed with EM at 40 days had lower P4 plasma levels than pregnant buffaloes since day 10 till day 20 after AI and similar to P4 levels of non pregnant buffaloes.
In another study, pregnant buffaloes showed higher milk whey concentrations of P4 than both animals showing embryonic mortality and non-pregnant buffaloes on day 20 and day 25 but only than non-pregnant buffaloes on Day 10 *(Campanile et al., 2007b).* These data let to hypothesize that embryonic mortality in buffalo species could be primarily due to a reduced P4 secretion by corpus luteum. This conclusion would be consistent with several findings in cattle and sheep, where early embryonic mortality was associated with reduced blood levels of P4 *(Garret et al., 1988; Mann and Lamming, 1999; Mann and Lamming, 2001).*
In a study carried out in Italy in a period of increasing daylight length it was hypothesized that the relatively high incidence of buffaloes with low P4 plasma levels after oestrus synchronisation was the result of a reduced activity of the reproductive endocrine system *(Zicarelli L.,1997).*
Impaired P4 secretion *(Wathes et al., 1998)*, however, has been found to be linked with a reduced capacity of the developing embryo to secrete the bovine trophoblastic protein-1 (bTP-1), also called Interferon-tau (IFN_; *Roberts et al., 1992)*, whose production at threshold amounts is responsible of one of the main mechanism of blocking luteolysis since day 16 post-AI *(Mann and Lamming, 1999)*
This protein is able to avoid corpus luteum regression *(Plante et al., 1989)* by two mechanisms: i) suppressing estradiol receptor and oxytocin receptor genes *(Spencer and Bazer; 1996; Robinson et al., 1997)*; ii) attenuating the endometrial secretion of PGF2_ *(Helmer et al., 1989a; Helmer et al., 1989b; Danet-Desnoyers et al., 1994)* and activating a prostaglandin inhibitor *(Thatcher et al., 1995).*
Oestradiol seems to be another factor involved in the luteolytic process, either by promoting OTR development and by stimulating prostaglandin secretion *(Wathes et al., 1998).*
In fact, in the ewe the number of oestradiol receptors on endometrium has been found significantly lower in pregnant vs not pregnant animals *(Lamming et al., 1995; Spencer et al., 1995).* However, in buffalo no difference were found in oestradiol plasma levels on day 0, 10, 20 and 25 after AI among pregnant, not pregnant and buffaloes undergone EM *(Spagnuolo et al., 2007).*
Gametes quality is another one of the main factors involved in EM event in domestic animals. Oocyte quality is able to affect embryo development and interfere with the following gestation. In buffalo species this could be involved in EM occurrence during the seasonal anoestrus, which coincides with day length increase. Campanile *et al. (2005)* demonstrated that 51% of buffaloes which showed EM had P4 levels on days 10 and 20 similar to those found in pregnant animals, showing that other factors, rather than reduced circulating P4 concentrations, also contributed to EM occurrence. Abdoon *et al (2001)* found that oocyte quality, defined as the capacity to result in embryonic development and pregnancy, was impaired in buffaloes during the anoestrous period occurring when daylight length increases *(Zicarelli, 1997).*
Furthermore, the incidence of EM between 40 and 60 days post AI is three times higher in buffaloes that had been acyclic at 70 days post partum *(Zicarelli, 1994)*, compared to the cyclic ones. It is known that in buffalo, a high incidence of oocyte atresia has been found and the mean recovery of good quality oocytes per ovary was low *(Gasparrini, 2002).* Maturation and quality of oocytes are influenced by granulosa cells that are sensitive to oxidative stress *(Dharmarajan et al., 1999)*. The antioxidant defence system plays a key role in preventing apoptosis and atresia, thus preserving steroidogenic function of granulosa cells *(Cassano et al., 1999).* Spagnuolo *et al (2007)* found no significant differences in redox status among pregnant, not pregnant and cows with embryonic mortality.

Hormonal treatments to reduce EM in buffalo.
The importance of P4 concentration during the first weeks of pregnancy on EM has been demonstrated in cattle *(Mann and Lamming; 1999 and 2001).* According to some reports *(Starbuck et al., 1999; Mann, 2002)*, the presence of an early P4 peak, within 5 days after mating or AI, facilitates the elongation of the conceptus and, consequently, the secretion

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of adequate levels of IFN_.
Several approaches have been used to increase P4concentration in blood in order to reduce the occurrence of EM. Increased P4 plasma levels were achieved either by inducing increased endogenous secretion or by administering exogenous P4 *(Mann and Lamming; 1999).* Several studies showed that the administration of natural sequence GnRH, GnRH agonists or hCG after AI can stimulate corpus luteum function, induce accessory corpus luteum formation, increase P4, and reduce estradiol production, with a consequent positive effect on embryonic survival *(Kerbler et al., 1997; Thatcher et al., 2003; Bartolome et al., 2005).* In buffaloes, hormonal treatments to reduce EM showed different results. Campanile *et al. (2007a)* reported that treatment with exogenous P4 (PRID®, Vetem) in buffaloes on day 5 after A.I gave low pregnancy rate and high incidence of EM, suggesting that exogenous P4 could have a detrimental effect on conception by inducing a down regulation of LH secretion and consequently reducing the capacity of the preformed corpus luteum to increase P4 synthesis and release. However, Kumar *et al. (2003)* reported an increase in conception rate in buffaloes treated with 125 mg of 17-_-_hydroxyprogesterone caproate s.c. on Day 4 after AI.
The injection of 12.6 µg GnRH agonist (Buserelin) or 1500 I.U. of hCG on day 5 after AI significantly increased P4 concentrations only at 10 days from treatment without reducing the incidence of EM. The lack of an effects on EM could be ascribed to the short lasting of P4 peak unable to have any influence on uterine function and embryo-maternal interactions *(Campanile et al., 2007a).* The P4 raise after the hCG injection was, however, ascribed by the authors most to an induced ovulation and formation of an accessory corpus luteum and not to the enhancing P4 secretion of the existing corpus luteum while both the effects has been reported in cattle *(Kerbler et al., 1997; Schmitt et al., 1996; Santos et al., 2001).* Treatment performed in buffaloes on Day 25 after AI with 341 mg of 17-_-_hydroxyprogesterone caproate administered i.m. 3 times, at 4-day intervals, reduced the incidence of EM in a buffalo herds characterised by a high incidence of embryonic mortality *(Campanile et al., 2007b).* Buserelin or hCG administration on Day 25 after AI in pregnant buffaloes also reduced the incidence of EM in a farm characterized by high EM occurrence *(Campanile et al. 2007b).* hCG administered at day 25 after AI induced ovulation in about 57% of buffaloes *(Campanile et al., 2007c)* and a similar response was found with GnRH agonist administrations, using which ovulation rates of 62% *(Campanile et al., 2007d)* and 68.6% *(Campanile et al., 2007c)* were observed, respectively after administration on day 5 or 25 post AI.
The mean follicular diameter sensitive to the hormonal treatment was about 8.9 mm in both GnRH and hCG treatments, varying between 4.2 and 13.0 mm *(Campanile et al., 2007c; Campanile et al., 2007d).* It is worth pointing out that the mean diameter of the follicles responsive to the treatments in buffaloes were similar to that of buffaloes in which ovulation did not occur and these data are in accordance with those reported in cattle *(Martinez et al., 1999).* Buffaloes that ovulated in response to the treatment with a GnRH agonist showed a progressive increase in milk whey P4 concentrations on days 10, 15 and 20, while P4 levels was relatively constant for buffaloes that did not ovulate. The injection of a GnRH agonist on day 5 after AI increased milk whey P4 concentrations in 97% of buffaloes subsequently pregnant on day 40, compared to 68% in the non-pregnant buffaloes ($P<0.01$). A greater ($P<0.05$) proportion of the buffaloes that ovulated (96.7%), compared to buffalo that did not ovulate (68.4%) were pregnant at 40 days after AI *(Campanile et al., 2007d).* Ovulation also increased milk whey P4 levels and reduced embryonic mortality in buffalo cows treated with 1500 I.U. of hCG or 12.6 µg of GnRH agonist on day 25 after AI *(Campanile et al. 2007c).*

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THE PREGNANCY-ASSOCIATED GLYCOPROTEINS: BIOCHEMICAL ASPECTS AND CLINICAL APPLICATION FOR PREGNANCY FOLLOW-UP IN BUFFALO (Bubalus bubalis)

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SUMMARY

Pregnancy diagnosis is an important part in reproduction management of ruminants. In the last years, a large polymorphic family of placenta-expressed proteins has been discovered in ruminant species and used for pregnancy diagnosis. Members of this family are named pregnancy- associated glycoproteins (PAG), being synthesized in the mono- and binucleate cells of the ruminant's trophectoderm. Part of them are released in the maternal blood circulation where they can be assayed by different laboratory techniques. Due to large variety of expressed molecules and to large variations in the post-translational processing of the PAG, different immuno-systems present different ability to quantify the PAG released in

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blood. The sensitivity (92 to 100%) and specificity of PAG radioimmunoassay when used for pregnancy diagnosis are very high. The assay of PAG in the buffalo cows can also bring very interesting information for researchers working in programs focused on the study of embryonic and fetal mortalities, as well as on embryo biotechnology (ET, FIV, clonage), animal nutrition, or infections diseases resulting in pathologies affecting the pregnancy.

INTRODUCTION

Placenta is a polyvalent organic system with a vital biological role for the perpetuation of the eutherian mammals. Fetal development is related to that of the placenta from the anatomical, genetic and metabolic points of view. As far as metabolism is concerned, endocrine function is particularly important. The endocrine function of the mammalian placenta includes various hormones (progesterone, estrogens, chorionic gonadotropins, placental lactogens, growth hormones…), and a series of growth factors and glycoproteins interfering with the establishment of pregnancy, corpus luteum maintenance, immunotolerance of the conceptus by the mother, intermediate maternal metabolism, fetal growth and mammary growth.
The study of the pregnancy-associated glycoproteins in ruminant species will be discussed below. Emphasis will be given to radioimmunoassay methods used for pregnancy diagnosis and for follow-up of placenta secretory function in different physiological or pathological conditions.

1. The pregnancy-associated glycoproteins (PAGs) in ruminant species

Characterized in the last 25 years *(Butler et al., 1982; Beckers et al., 1988a,b; Zoli et al., 1991; Xie et al., 1991)*, they constitute a large family of glycoproteins expressed in the outer epithelial cell layer (chorion/ trophectoderm) of the placenta of eutherian species. They are synthesized by the mono- and binucleate thophoblastic cells, some of them being secreted in maternal blood from the moment when the conceptus becomes more closely attached to the uterine wall and formation of placentomes begins *(Zoli et al., 1992a; Wooding, 1992)*.
By using biochemical procedures, some molecules of the PAG family were isolated from cotyledons of cow *(Zoli et al., 1991; Sousa et al., 2002; Klisch et al., 2005), ewe (Xie et al., 1997a; El Amiri et al., 2003, 2004), goat (Garbayo et al., 1998)*, buffalo *(Barbato et al., 2006)*, bison *(Kiewisz et al., 2007)*, moose and elk *(Huang et al., 1999)*. Purified and semi-purified preparations were used to immunize rabbits and the antisera (AS) obtained allowed the development of homologous *(Sasser et al., 1986; Humblot et al., 1988; Zoli et al., 1992b; Mialon et al., 1993)* and heterologous radioimmunoassay (RIA) *(Ranilla et al., 1994; González et al., 1999; El Amiri et al., 2006; Ayad et al., 2007)*, and sandwich enzyme-linked immunosorbant assay (ELISA) systems (Green et al., 2005).
Screening of placental libraries with nucleic acid probes has identified additional (probably more than 100) cDNA that code for PAG molecules *(Xie et al., 1997b; Green et al., 2000; Garbayo et al., 2000)*. Recent investigations have also demonstrated that different PAG cDNA are not expressed coordinately throughout pregnancy *(Green et al., 2000; Hughes et al., 2000)*. Some, for example, are expressed early, others only when pregnancy progresses. An important general feature of ruminant PAG is that they are extensively glycosylated proteins undergoing a complex post-translational processing, with the carbohydrate signature of the trophoblastic cells *(Klisch et al., 2006)*. Apparent molecular masses of purified PAG showed a major variability, having higher estimated values (55 to 70 kDa) than the expected molecular mass of their protein core (37 kDa) *(Klisch and Leiser, 2003; Klisch et al., 2005)*. The variable degree of glycosylation in the different PAG has been claimed to be an important factor regulating plasma half life of these proteins including their peripheral concentration *(Klisch et al., 2005)*. Interestingly, the presence of antigens immunologically similar to boPAG67kDa has also beendemonstrated in testicular tissue and in ovarian extracts *(Zoli et al., 1990a,b)*, justifying the adjective “associated” and not “specific” given to this glycoprotein. However, at our bestknowledge, no further investigation was made in this way and so, the hypothetical synthesis of a molecule related to PAG in the Sertoli cells and ovarian tissue was not confirmed experimentally. No precise function could be experimentally demonstrated for PAGs molecules *(Green et al., 1998; Wooding et al., 2005)*, however, their high level of expression in early gestation *(Ushizawa et al., 2004, 2005)* point to a fundamental role of such as molecules in implantation and placentogenesis *(Ishiwata et al., 2003)*.

2. Influence of different factors on PAG profiles

PAG concentrations have been directly

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correlated to the placental mass *(Vasques et al., 1995; Patel et al., 1997; Wallace et al., 1997)*, which in turn is related to the stage of pregnancy *(Wooding, 1993; Ferrel, 1991)*. Investigations realized in peripartum clearly demonstrated a positive influence of both maternal environment and fetal genotype (sex and race) on peripheral blood concentrations of PAG molecules *(Zoli et al., 1992b; Guilbault et al., 1991; Kornmatitsuk et al., 2002)*. Higher PAG concentrations are observed in maternal than in fetal serum, suggesting that this glycoprotein is delivered preferentially in the maternal system *(Zoli et al., 1992b)*. Another original approach was recently developed by Lopez-Gatius et al. *(2007)*, who determined plasma levels of bovine PAG (measured by both RIA-497 and RIA-706) and progesterone in Holstein Friesian dairy cows. Cows were followed by venipuncture on Days 35, 42, 49, 56 and 63. The results showed an interaction between the milk production and the PAG levels measured by both RIA systems. PAG concentrations decreased when dairy milk production increased whereas the progesterone levels remained unaffected by milk production. It remains to be determined if the PAG decrease is due to an increased traffic of PAG to the mammary gland or to an increased clearance due to a higher metabolic activity.

3. Pregnancy diagnosis

In practice, the measurement of PAG concentrations in peripheral maternal circulation has been used fort both pregnancy confirmation and the follow-up of the trophoblastic function.
The first aspect can help veterinarians and breeders in the management of reproduction, while the second represents a powerful tool for investigators involved in studying factors affecting embryo and fetal mortality and embryo biotechnology. In the field, the measurement of PAG was developed for routine analysis in veterinarian laboratories. The leader country is France with almost 100,000 pregnancy diagnoses per year, followed by Belgium (6,000) and other European countries such as Spain and Scotland. Presently, PAG measurements are also starting at the University of Perugia (Italy).

4. Clinical application of PAG RIA for pregnancy follow-up

The assay of PAG can also bring very interesting information for researchers working in programs focused on embryonic and fetal mortalities, on embryo biotechnology (ET, FIV, somatic nuclear transfer), on animal nutrition, and in infections diseases. Some aspects of PAG concentrations at different pathological conditions will be described below.

5. The pregnancy-associated glycoproteins (PAGs) in buffalo cows *(Bubalus bubalis)*

The world's buffaloes are classified in two groups: the African and the Asian. The two groups have been assigned different generic names: Syncerus and Bubalus. African and Asian buffaloes are from different lineages, separated from the other bovines during the Pleistocene *(Savage and Russel, 1983)* [1] or even late Miocene *(Hassanin and Douzery, 1999)*. The African buffalo, Syncerus, consists of a single species, Syncerus caffer, with a small number of sub-species. The Asian buffalo, Bubalus, includes three species: the anoa of Celebes, the tamarao of Mindoro and the arni or Indian wild buffalo *(Tanaka et al., 1996)*. Indian wild buffalo (Bubalus arnee) was the only domesticated species, and therefore has been given the species name bubalis. It is common throughout Asia. It was also introduced into different parts of Europe, Russia and South America. In Northeast Asia and China, buffaloes are of the Swamp type (2n = 50), mainly used for work but becoming increasingly important for meat production. Buffaloes from India and Pakistan, as well as those from Europe, are mostly of the River type (2n = 48), usually large in size and noted for their milk production *(Mahadevan, 1992)*.
Recently, Barbato et al. *(2008)* have described the first isolation and characterization of PAG from buffalo placenta. Three distinct wbPAG sequences were identified and deposited in the SwissProt database: RGSXLTIHPLRNIRDFFYVG (acc.no. P85048), RGSXLTILPLRNIID (acc.no. P85049) and RGSXLTHLPLRNI (acc.no. P85050). Their comparison to those previously identified revealed that one of them was identical to both boPAG-4 and ovPAG59kDa, while the others appear to be new since they have not yet been described.

PAG radioimmunoassay procedure
Three mature New Zealand white rabbits (AS#858, AS#859 and AS#860) were immunized with distinct purified PAG preparations by intradermal route *(Vaitukaitis et al., 1971)*. For the first immunisation, 300 μg

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of proteins were dissolved in 1.0 mL phosphate buffer 0.5 M (pH 7.5) and emulsified with Freund complete adjuvant (Difco Labs., Detroit, MI). Booster doses (300 μg) were injected at 3-4 week interval (Freund incomplete adjuvant). Blood was collected from the marginal ear vein starting one month after the first injection and then once a month. Blood samples were allowed to clot overnight at room temperature. Thereafter, they were centrifuged at 1,000_g for 20 min, and the sera were stored at –20 °C until used. The immunization protocol was approved by the Animal Ethics Committee of the University of Liege (Dossier number 287).

In the presence of excess antibody, 44% (AS#858), 45% (AS#859) and 40% (AS#860) of labelled bovine 67 kDa PAG (boPAG67kDa) *(Zoli et al., 1991, 1992)* were bound. These antisera were tested at different dilutions to obtain a tracer-binding ratio in the zero standard of approximately 20% (B_0/Tc) and a low NSB (< 1%). The optimal binding ratios were obtained at initial dilutions of 1/350,000 (AS#858), 1/640,000 (AS#859) and 1/840,000 (AS#860). The antiserum giving the highest dilution titer (AS#860) was used for PAG-RIA development.

The PAG measurements were performed according to the method described by Zoli et al. *(1992)* with some modifications.

Several methods were used for pregnancy diagnosis in cows and buffaloes, including rectal palpation *(Oltenacu et al. 1990; El-Battawy 1993)*, ultrasound *(Beal et al. 1992; Brito et al. 2002)* and a milk progesterone assay *(Oltenacu et al. 1990; Sharma et al. 1990)*. Although PAG profile has been described for many species, information about the levels in buffalo cows has not been reported. The knowledge of this profile is of fundamental importance for further physiological investigations and practical applications.

By using homologous RIA system, the mean PAG concentration observed in pregnant buffalo females at Day 23 after breeding is 1.45 ± 0.48 ng/mL Concentration increased gradually until Day 80. However, it was surprising to find that PAG concentrations in pregnant buffalo cows were almost 2 to 3 times higher than those observed in dairy cows from Day 30 till 60 after breeding *(Perenyi et al. 2002b; Lopez-Gatius et al. 2007)*. A rapid increase on PAG concentrations associated with high maternal concentrations at early pregnancy period are characteristic of caprine *(Gonzalez et al. 2000)* and ovine species *(Ledezma-Torres et al. 2006)*. On the opposite, in cattle, concentrations increase slowly and remain at low levels during early pregnancy gestation *(Zoli et al. 1992b; Patel et al. 1997; Perenyi et al. 2002a)*. In the post partum period, the concentrations of PAG in buffalo cows decreased gradually until Day 42 (0.2 ng/mL).

CONCLUSION

Thanks to international collaborative studies, we have shown that PAG levels are a good indicator of feto-placental well-being and that sharp decreases in PAG levels occur just before pregnancy failure in cows as well as in other ruminant species and in buffalo also. In some countries, the PAG assay is available for Doctors in Veterinary Medicine in the regional laboratories responsible for animal health including immunodiagnosis for brucellosis, IBR, BVD, CAEV, VISNAMEDI etc. The isolation of new antigens from buffalo placenta allowed the production of several antibodies against buffalo PAGs.This production is useful in developing homolougous RIA and EIA technique currently used for pregnancy diagnosis and physiopathological investigations (...embryo mortality) in farm animals. The production of specific antisera can be very useful in immunohistochemical and immunocytochemical studies of PAG expression in fetomaternal interfaces.

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IMPROVEMENT OF PRODUCTIVE AND REPRODUCTIVE EFFICIENCY OF ANATOLIAN BUFFALOES THROUGH THE USE OF ARTIFICIAL INSEMINATION WITH ITALIAN BUFFALO BULLS SEMEN

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ABSTRACT

This experiment was designed to determine suitable buffalo genotype for Hatay Region of Turkey by using semen from proven Italian River Buffalo bulls at Ilıkpınar Village of Kırıkhan District of Hatay Province of Turkey. The material of the research was consisted of breedable Anatolian buffalo cows and their progenies. Buffalo cows from Anatolian breed are being inseminated with the semen of Italian River buffalo bulls artificially after being synchronized of estrus. Crossing will be continued up to G1 genotype level. During the experiment productive, reproductive, growth performances, milk composition, in addition beginning from Au_ust 2006 rennet coagulation time, somatic cell count are being determined on Anatolian, F1 and G1 genotypes. Until March 2008, as a total of 105 crossbred buffaloes (65F1, 5G1,7F2,28 F1 x Anatolian) were born in the Project. From now on the Project will continue approximately up to 2010. At that time it will be reaced to efficient number F1 and G1 materials, and it will be tried to become fixed G1' characterisitcs at Ilıkpınar environmental conditions.

Keywords: Genetic improvement, artificial insemination, Anatolian buffalo, Italian Buffalo.

INTRODUCTION

Detect of oestrus and ovulation are difficult in buffaloes, because of clear slightly oestrus thanks to that application of artificial insemination is not easy *(Ohashi, 1994, Seren et al, 1995)*. There are significant variation in duration of heat (4-64 hours) *(Baruselli, 2001).*
Various heat synchronization protocols were

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evaluated for oestrus synchronization and ovulation in buffaloes. It is ideal that the protocols for synchronization and ovulation have to be effective, not only in syclic buffaloes, but also non-syclic ones and when the protocols are combined with suitable artificial insemination, an high conception rate have to be obtained. Various literature knowledge on conception rate obtained in the end of various syncronization and insemination protocols are shown in *Table 1*.
The Project was realised as an outcome of Cairo Meeting that hold in 1996 of Interregional Research Network on Buffalo of FAO. It was started at April of 2002 at Ilıkpınar Village of Kırıkhan District of Hatay Province. The Project, as an colloborative Project of 2 countries (Turkey and Italy) was come in to force by using semen originated from 2 Italian bulls which was provided by FAO. At 2005 aids (900 doses semen and chemicals for 80 buffalo cows) provided by FAO were exhausted, in order to continue to the Project financial support was taken from The Scientific and Technical Research Council of Turkey (TÜB_TAK) for 15 April 2006-15 April 2007 period. So, we purchased 600 doses buffalo semen from 4 bulls.
This study was designed to determine suitable buffalo genotype for Hatay Region of Turkey by using semen from proven Italian Buffalo bulls at Ilıkpınar Village of Kırıkhan District of Hatay Province of Turkey.

MATERIAL AND METHODS

The material of the research was formed by various data of Anatolian and Anatolian x Italian crosbred F1 buffaloes belong to buffalo herd of Ilıkpınar Village.It can be said that, feeding is almost based on Village pasture. Some extra food are given in the evening at the units. At the beginning (2002), 8 units were included in the Experiment. But, Experiment have been continued from 2002 until now in 3 units. Because of, buffaloes in other units in the Village were leaved out of the experiment in various years beginning 2003, because of cooperation could not be continued with the farmers. Feeding and care possibilities are well in one of the 3 units (1st), but other two of them, it is poor.
Multiparous buffalo cows after being examine the cows, which had not pregnant and had not any reproductive problem, had received an drug release device (PRID) intravaginally. PRID were kept for 10 days in uterine. On the 7th day after PRID insertion, an injection of 0.15 mg cloprostenol is given. Because of buffaloes were non-cyclic also 1000 I.U. Pregnant mare serum gonadotrophin (PMSG) were given. Cows were artificially inseminated at 48, 72 and 96 hours with 2 doses semen after the removing of PRID. The cows, which were not heated during both of insemination times, were accepted estrus synchronzation was been unsuccesful. The protocol that we applied in last four years as follows (because of exhausting of Dalmazin that had been provided by FAO); 2 cc estrumate (PGF2alpha) injection after 7 days from PRID insertion intravaginally, PRID removing after 10 days from its application, 2 cc dalmarelin (GnRh) injection and insemination artificially at 48, 72 and 96 hours with 2 doses semen after removing PRID.
Classification of semen of bulls to cows were realized randomly. Until 30 days after insemination buffalo cows were kept in the barns, because of however most of the bulls are F_1, but some of them are Anatolian on the village pasture. Pregnancy diagnosis was assessed at 90 days from the insemination by rectal palpation of uterine contents. Pregnancy rates were calculated for animals that were pregnant at 90th days. Calving dates of pregnant cows are estimated, by assuming of pregnancy period as 310±15. It is accepted that the cows, that calved after 20 days from estimated date, coud be synchronized of their oestrus, but, they

Table 1. Various literature knowledge on conception rate obtained.

PROTOCOL	RESEARCHERS	CONCEPTION RATE (%)
PRID+1000 IU PMSG	Zicarelli et al, 1997	29.9
PRID+PMSG+PGF2_	Barile et al (1997)	34.8
PRID	Barile et al, 2001	51
PGF2_	Neglia et al, 2001	45
PGF2_+ GnRH	"	48.8

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conceived in the pasture (with Anatolian or F_1 bulls). Calves can be estimated if they have Anatolian or F_1 sires by make use of shape of their noise structure and their behavior to the own owner.
Because of feeding is based on the pasture, increasing of numbers of F_1 and G_1 animals are slow. In addition animals in F_2 and Anatolian x F_1 genotypes are also born. But, the determinations are also done for F_1's and G_1's.
Inseminations have been continued 2002, 2003, 2004, 2005, 2006, 2007 and 2008 years. For the last time (April 2008), 23 buffalo cows, that 12 of them are F_1, were inseminated. So, as a total of 184 buffalo cows inseminated artificially.
Measuring body measurements in 1, 3, 6, 9 and 12 month-ages of the calves that were born in TÜB_TAK support period and monthly milk tests were continued. Total dry matter (TDM), solid non fat (SNF), protein analyses were done in the milk samples of Anatolian and F_1 cows which were taken in the morning milking of milk test days. In addition beginning August 2006, somatic cell score could also be possible in the same samples.
Fattening was applied on 3 F_1 and 6 Anatolian male calves which they were in almost the same ages. In addition various body measurements and live weights were determined of all the calves on not only project's material, but also their contemporaries from Anatolian breed, and in addition on the monthly milk test days milk samples have been taken individually, and milk analyses have been performed for milk components and somatic cell count (SCC). Protein and fat contents were determined by Formol Titration *(James, 1998)* and Gerber Methods *(Kurt, 1984)* respectively.
The Project will also continue from 2008 approximately up to 2010. At that time it will be reaced to efficient number F_1 and G_1 materials, and it will be tried to become fixed G_1' characterisitcs at Ilıkpınar environmental conditions.

RESULTS

Italian crosbred buffaloe numbers which were born in 2002-2008 period and the averages of various characteristics are shown in *Table 2* and *Table 3* respectively.
In the Project until March 2008, as a total of 105 crossbred buffaloes (65F_1, 5G_1, 7F_2, 28 F_1xAnatolian) were born *(Table 2)*.
Until May 2008 ten F1 buffalo cows (two of then 2 times) calved. And at that time 8 lactation (1st) were completed. In one unit that feeding conditions are the best, and 5 first lactations were completed. In this unit lactation average is 1386.2 lt. General average, 8 units together, is 1203.0 lt. Lactation yield average of 81 Anatolian buffaloes are calculated as 961.59±342.13 kg.

Table 2. Italian crosbred buffalo numbers which were born in 2002-2008 period.

GENOTYPE	SEX		TOTAL
	Male	Female	
F_1	36	29	65
G_1 (Italian x F_1)	3	2	5
F_1	2	5	7
F_1 x Anatolian	12	16	28
Total	53	52	105

DISCUSSION

Until now 6 times (in the May mounts of 2002, 2003, 2004, 2005, 2006 and 2007) inseminations were applied. 50.0%, 55.2%, 57%, 52% (Şekerden et al, 2003), 42.2% and 61.5% *(Anonymous, 2007)* pregnancy rates (at only first inseminations after being synchronized) were obtained in 2002, 2003, 2004, 2005, 2006 and 2007 inseminations in Ilıkpınar Village herd. In the April 2008, 23 buffalo cows, that 12 of them are F_1, were inseminated. It is interesting, it was determined that 5 of 23 buffaloes were conceived. It means that conception rate is 78% in the last insemination time.
Because of In Turkey artificial insemination first time applied, the rates were perfect compared with the results obtained in other countries which had applied the same synchronization protecol *(Baruselli et al.,1997; Zicarelli et al., 1997; Barile et al., 2001; Neglia et al., 2001).*
Lactation milk yield of F_1's (general average) is higher 25.18 % than Anatolian cows. But for only the unit that have good feeding possibilities is higher 44.18 % than Anatolian *(Table 3)*. In addition, the lactation milk yields of F_1 are belong to 1st lactation order, although the milk yield average of Anatolian cows were calculated from 81 various lactation (1st-6th) milk yields, and the average of F_1 lactation milk yield was calculated by using 8 first lactations milk yields.

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Table 3. The averages of various characteristics (x).

CHARACTERISTICS	GENOTYPE				
	Anatolian		F_1 (Anatolian buffalo cow x Italian buffalo bull)		
	N	$\bar{X} \pm S_{\bar{X}}$	N (xx)	$\bar{X} \pm S_{\bar{X}}$	Superiority (%)
305-day milk yield (kgs)	81	961.6±342.13	5	1386.2±282.84	44.2
Daily milk yield (kgs)	81	3.1±1.12	5	4.52±0.92	45.8
Fat %	81	7.2±0.82	5	6.18±0.02	-14.1
Protein%	53	4.74±0.91	5	3.12±0.65	-34.1
TDM%	81	17.1±0.81	5	15.61±0.11	-8.71
SNF%	17	9.7±0.76	5	10.64±0.17	9.7
Lactose%	19	5.2±0.39	5	5.27±8.485E-02	1.34
SCC (μ/lt)	17	230±120	5	69±120	70

(x) Data obtained in April 2002- 15 April 2008 period were evaluated together. (xx) Data are belong to only 1st unit and 1st lactation milk yield.

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PLASMA INHIBIN-A IN BUFFALO COWS: RESPONSES TO THREE SYNCHRONIZATION TREATMENTS AT PERIOVULATORY PERIOD

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ABSTRACT

To test whether Inhibin-A assays can be used to predict the fertility in buffalo cows, 15 buffaloes were assigned to 3 synchronization treatments: group A (n=5) treated with PRID for 10 days+1000 IU PMSG and PGF2_ (0.15mg cloprostenol) on the 7th day; group B (n=5) treated as group A but PMSG and PGF2_ were administered on the 10th.day; group C (n=5) received GnRH (150_g gonadorelin) on day 0 + PGF2_ on the 7th day + GnRH on the 9th day. Buffaloes were artificially inseminated at 72 and 96h from PRID removal in group A and B and at 40h from the 2nd GnRH injection in group C. Starting 2 days (-2d) before the 1st insemination the number and size of all follicles>2mm were assessed for 4 days by ultrasound and plasma Inhibin-A levels were measured. The conception rates were 80%, 40% and 20% in groups A, B and C, respectively. In all groups there was a positive correlation between number of follicles>6mm and Inhibin-A (r=0.92, P<0.0001)assayed two days before the 1st insemination; a positive correlation between the same parameters (r=0.97,P<0.0003) assayed the same day
(-2d) was found only in pregnant buffaloes. In the same day (-2d) the Inhibin-A levels were 29.6, 9.9 and 6.5 pg/ml (P<0.05) in groups A, B and C, respectively and 21.5 and 9.9 pg/ml in pregnant and non pregnant buffaloes. These results suggest that follicles>6 mm are the main source of Inhibin-A and this latter could be useful to predict the outcome of artificial insemination.

INTRODUCTION

Inhibins are gonadal peptides that selectively and potently inhibit FSH secretion from the pituitary gland *(De Jong, 1988; Ying, 1988)*. They are composed of an α-subunit and one of two β-subunits (βA or βB), with α-βA and α-βB dimers forming Inhibin-A and Inhibin-B, respectively. The importance of Inhibins in the control of the reproductive function has been reported *(de Kretser et al., 2002, Medan et al., 2007)* and it appears that βB-subunit might be produced in small developing follicles, which is re-placed by the Inhibin βA-subunit as the follicles approach the preovulatory stage *(Medan et al., 2007)*. In cattle *(Kaneko et al., 2002)* and goats *(Medan et al., 2005)* an inverse relationship between FSH and Inhibin-A was demonstrated, suggesting the key role of Inhibin-A produced by dominant follicle(s) in terminating the transient peaks of FSH secretion *(Medan et al., 2007)*. Following the isolation of Inhibin-A and Inhibin-B, a large number of studies have defined a variety of physiological roles for these substances that range well beyond their ability to suppress or stimulate follicle-stimulating hormone (FSH) secretion. Using an ELISA assay that have now been shown to detect not only dimeric Inhibin but products of the α-subunit, changes in Inhibin-A secretion have been identified in a variety of patho-physiological states related to reproductive functions. Evidence is emerging that monitoring the stimulation phase of assisted reproductive technologies with Inhibin-A as well as ultrasound scans give a good indication of follicular recruitment and development. Very few data are available on the Inhibin-A plasma concentrations in buffaloes during reproductive treatments *(Palta et al., 1997)*, so this our preliminary paper reports results on the relationship between Inhibin-A levels, follicular development and conception rate in three different synchronization treatments in adult buffalo cows.

MATERIAL AND METHODS

The trial was carried out during the non-breeding season on 15 buffalo cows divided in three homogeneous groups: Group A (n=5) treated with a progesterone releasing intravaginal device (PRID) inserted for 10 days + an i.m. injection of 1000 IU of PMSG and 0.15 mg of cloprostenol ($PGF2_a$ analogue) on day 7; Group B(n=5) treated as in Group A but

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received the PMSG and $PGF2_a$ on day 10 (day of PRID removal) instead on day 7 (Day 0= day of PRID insertion); Group C (n= 5) treated with Ovsynch protocol and received i.m. injection of GnRH on day 0 + $PGF2_a$ on the 7th day + GnRH on the 9th day (GnRH=μg150 gonadorelin; $PGF2_a$=0.15mg cloprostenol). Buffaloes were artificially inseminated with frozen/thawed semen of progeny testing bulls at 72 and 96 h from removal in the PRID groups (A and B) and at 40 h from the 2nd GnRH injection in the Ovsynch group (C). The pregnancy diagnosis was performed on day 26 after the first artificial insemination (AI) by an ultrasound sector scanner with a 7.5 Mhz rectal linear probe and confirmed by rectal palpation on day 45.

On day -2, -1, 0 (day 0 = day of 1th AI in group A and B; day of a single AI in group C) all buffaloes undergone an ultrasound examination of ovaries by using a portable ultrasound unit (Aloka SSD-500, Aloka CO. Ltd., Tokyo, Japan) together with a 7.5 MHz linear rectal probe. Follicles were classified in four categories according to their size: small (diameter < 3 mm), medium (3 mm< diameter <6 mm), large (6 mm< diameter <9 mm) and very large (diameter >9 mm). The number of all follicles and the number and the diameter of medium and large follicles were recorded each day.

At the same time of ultrasound examination blood samples were collected by jugular venipuncture with evacuated tubes containing K3 EDTA (Venoject, Terumo Europe NV, Leuven, Belgium), immediately centrifuged (2500 G for 15 min) and the plasma stored at -20°C until assayed. Inhibin-A concentrations were determined in the plasma samples by an enzimatically amplified "two-step" sandwich type immunoassay kit for human serum or plasma (Inhibin A DSL-10-28100, Diagnostic Systems Laboratories Inc, Webster, Texas, USA).

Data were analyzed by a standard statistical analysis (SAS, GLM Procedures).

RESULTS

On day -2 all animals had one large and very large follicle, except for one animal of group B that had only medium follicles; although the number of large and very large follicles was higher in group A than in group B and C, the differences were not significant. In group A ($P< 0.05$), B and C a reduction in the number of very large follicles was observed on day 0 *(Table 1)*.

Plasma concentration of Inhibin-A was significantly different ($P<0.05$, *figure 1*) among the three treatment groups on day -2 when the Inhibin-A levels were 29.6, 9.9 and 6.5 pg/ml in groups A, B and C, respectively. In buffalo cows resulting pregnant and non pregnant the Inhibin-A levels were 21.5 and 9.9 pg/ml, respectively.

Table 1. Follicles number among treatment groups within the categories observed: small (<3mm), medium (3<diameter<6), large (6<diameter<9), very large (>9mm).

Day	Group	N° animals	Follicles					
			Small	Medium	Large	Very large	Large + Very large	Total
Day -2	Group A	5	10.4a	1.7	2.0	1.6	2.4	13.8a
Day -2	Group B	5	6.8b	2.3	1.3	1.0	1.5	9.8b
Day -2	Group C	5	6.8b	2.3	1.5	1.0	1.2	9.8b
Day -1	Group A	5	10.2	1.0	2.0	1.4	2.2	12.8
Day -1	Group B	5	7.2	3.0	1.3	1.0	1.4	11.0
Day -1	Group C	5	7.4	2.0	1.0	1.0	1.0	9.2
Day 0	Group A	5	13.2a	3.0	2.0	0.4	0.8	15.8
Day 0	Group B	5	6.3b	4.0	1.5	0.5	1.4	9.6
Day 0	Group C	5	8.6ab	5.5	1.0	0.5	0.6	12.8

Day 0 = day of 1th AI in group A and B; day of a single AI in group C. Values within columns (day per day) with different letters differ for $P<0.05$.

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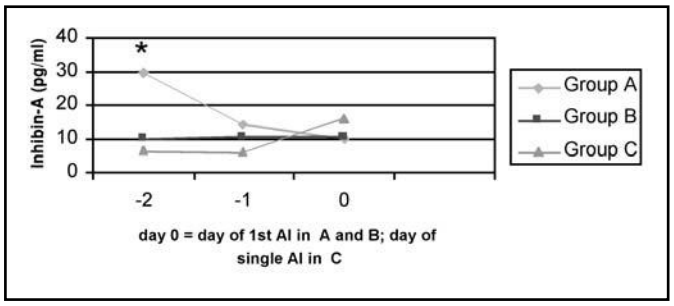
Figure 1. Mean peripheral plasma Inhibin-A concentrations relative to the artificial insemination



Means marked with an asterisk (*) are significantly different (P<0.05).

In all groups there was a positive correlation between number of follicles>6mm and Inhibin-A (r=0.92, P<0.0001) assayed two days before (-2d) the AI; a positive correlation between the same parameters (r=0.97, P<0.0003) assayed the same day (-2d) was found only in pregnant buffaloes. Time to ovulation tended to be shorter in association with higher Inhibin-A assayed two days before (-2d) the AI (r=-0.44; P<0.09) *(Welt et al., 1999).*

Time of ovulation was influenced by treatments: better results have been observed at the time of AI in group A where the overall ovulation rate was 100% (80% of animals was observed ovulated at the 1th AI and 20% at the 2nd AI); in group B the overall ovulation rate was 40% (20% of animals was observed ovulated at the 1th AI and 20% at the 2nd AI); in group C 60% of animals was observed ovulated at the time of AI. The overall conception rates were 80%, 20% and 40% in group A, B and C, respectively *(Table 2).*

CONCLUSION

This is the first report to describe the dynamic changes in plasma concentration of Inhibin-A in buffalo cows subjected to different synchronization protocols. The results suggest that follicles > 6 mm are the main source of Inhibin-A and day-2 peripheral Inhibin-A levels measured during different synchronization protocols could be a good marker for follicular development and pregnancy rate. A large prospective study is needed to confirm our findings.

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Table 2. Ovulation rate and conception rate among treatment groups.

Group	N° Animals	Ovulation rate % (n)		Conception rate % (n)
		At 1st A.I*	At 2nd A.I**	on day 26 after the first AI
Group A	5	80 (4/5)	20 (1/5)	80 (4/5)
Group B	5	20 (1/5)	20 (1/5)	20 (1/5)
Group C	5	60 (3/5)	--	40 (2/5)

* 1st AI = 72h from PRID removal in group A and B; single insemination at 40 h from 2nd GnRH injection in group C
** 2nd AI = 96h from PRID removal in group A and B.

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MILK FLOW TRAITS IN MEDITERRANEAN ITALIAN PRIMIPAROUS BUFFALO COWS

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ABSTRACT

The aim of this study is to evaluate milk yield and flow at morning and at evening in primiparous buffaloes. 208 milk flow curves from 26 primiparous buffaloes have been detected, with a portable milkmeter Lactocorder (WMB AG). Main milk flow traits were recorded. All the analyzed recorded curves showed, as final results, that the incresing phase (44,2%) predominated respect to the decreasing phase (30,7%) and to the plateau (25,1%). Significant differences between morning and evening were observed in the milk production (P=0,001), in the peak flow (P=0,001), in principal milking time (P=0,01) and in principal milking production (P=0,01). The produced milk in the first 3 minutes and the produced one during principal milking are the 70,3% and 87,0% of the total respectively. Principal milking represented the 64,7% of total milking time. At the end we observed a significant correlation between: decreasing phase and somatic cells (r=0,26); plateau phase and somatic cells (r=-0,23).

INTRODUCTION

Graphical representation in milk ejection is visible through flow curves. They are typical and characteristic for each species (buffalo, cow, sheep, goat, horse) and they are influenced by anatomical and environmental factors *(Thomas, 2004)*. The portable milkmeter Lactocorder (WMB AG - Balgach) is the instrument used to relieve real time milk flow curves and their parameters.
Milk flow curves have been well studied in the cow, partially in sheep and goat *(Didzic, 2004)* and a relatively recent approach is the introduction of this method in buffalo field. The graphic results are represented by 3 different phases and an eventual fourth one: the first is the increasing of milk flow, represented by the time elapsed between the attachment of the milking clusters and the time until constant milk flow; the second is the plateau with a constant milk flow (peak of milk flow is generally in this phase); the third is the decreasing phase and represents the time from the plateau phase until milk flow below <0,2 kg/min; an eventual fourth phase may be the blind phase (***figure 1***; *Boselli et al., 2008)*.

Milk flow curves and other parameters are influenced by anatomical, physiological and environmental factors. Mediterranean Italian buffalo is characterized by longer teat than in dairy cow (Weiss et al., 2004) and by a small cisternal fraction *(5%; Thomas et al., 2004a)*. These differences characterize the milk flow curves in buffalo species, as result in different relieved parameters as the milking time and milk yield. The aim of this study is to analyze the principal milking phases and milk yield in Mediterranean Italian primiparous buffalo cows, to correlate plateau and decreasing phase with somatic cell count (mastitis indicators).

MATERIAL AND METHODS

This study was carried on 26 primiparous buffalo cows in Tormancina Farm (Animal Production Research Centre of CRA) and on 208 milk flow curves (104 on the morning and 104 on the evining). Each buffalo was recorded every two months, during the whole lactation. Buffalo cows were milked at 6.30 AM and 5.30 PM in a Herringbone (8+8) milking room, the milk vacuum was set at 44 kPa, pulsation rate was 60 cycles/min, with a 60:40 pulsation ratio. Milk yield (kg/min), maximum flow (kg/min), yield at first 3 min (kg), principal milking time (min), average flow in principal milking (kg/min), yield in principal milking (kg), increasing phase (min), plateau phase (min), decreasing phase (min), total milking time (increasing + plateau + decreasing), somatic cell count (cell./ml) were measured. Individual milk samples were automatically collected by lactocorder and somatic cell parameter were evaluated by a Fossomatic 5000 (Foss Electric). Results are presented as mean ± s.d. and the statistical analysis were carried on by MedCalc®

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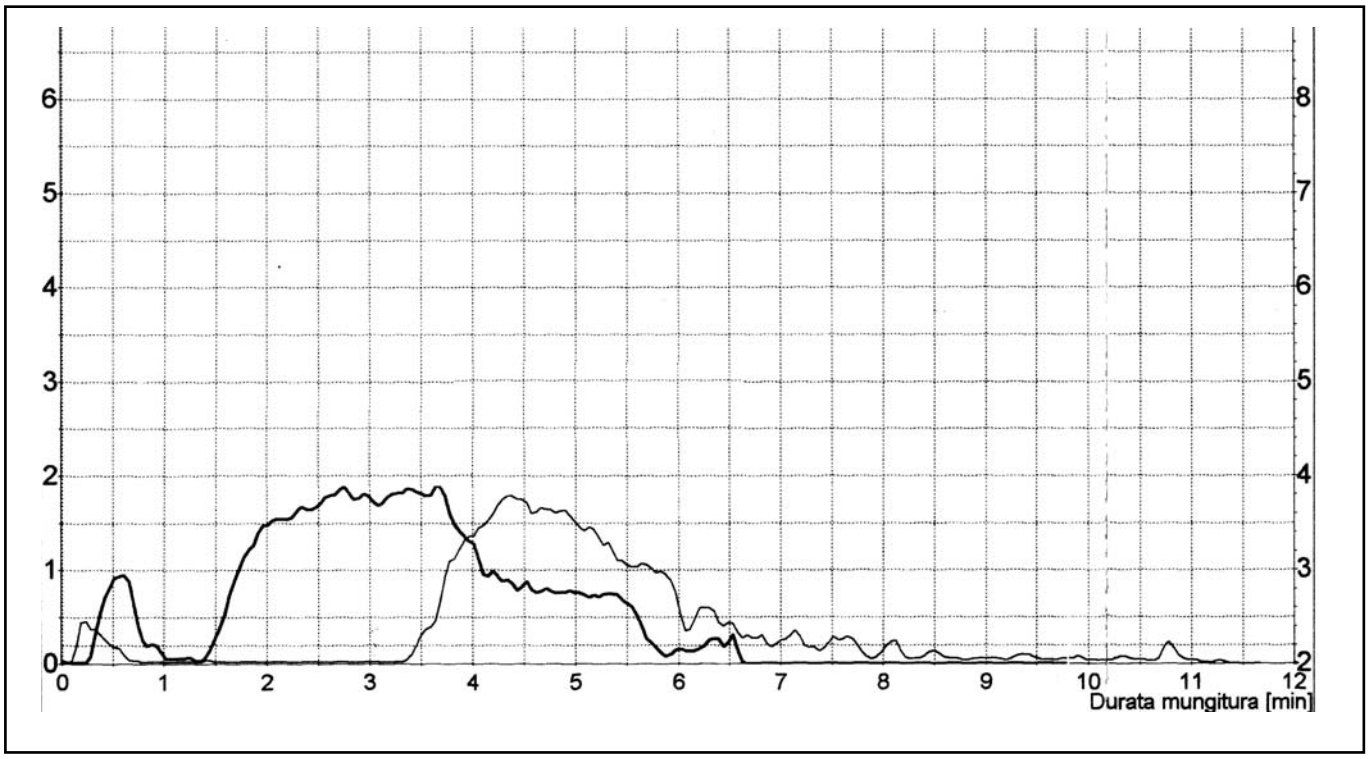
Figure 1. Morning (black line) and evening (grey line) milk flow curves with evident cisternal fraction, on the same buffalo.



v9.3.0.0, using t test to compare mean results.

RESULTS AND DISCUSSION

Significant differences between morning and evening were relieved in the milk yield, 4,63±1,98 kg vs 3,52±1,64 kg (P=0,001) and in the yield at first 3 min, 3,16±1,48 kg vs 2,58±1,22 (P=0,01). Principal milking time resulted longer in the morning than in the evening 4,30±2,11 min vs 3,54±1,84 min (P=0,01), but in this phase the production was the same: 87,9% (morning) and 86,4% (evening). Maximum flow and mean flow resulted significantly different, 1,56±0,59 kg vs 1,29±0,56 (P=0,001) and 0,99±0,39 kg vs 0,84±0,34 (P=0,01). The main relieved parameters are reported in **table 1**.
Significant differences were not observed between morning and evening in total milking time, 6,20±2,57 min vs 5,93±2,91 min, nor for the three main phases of the flow curves (increasing, plateau and decreasing). The analysis of all 208 recorded milk flow curves, showed that the increasing time (2,68 min, 44,2%) was longer than the decreasing time (1,86 min, 30,7%) and the plateau phase (1,52 min, 25,1%) as reported in **table 2**. This evidence is respected in morning and evening milking comparison. On the contrary, Sandrucci et al. (2007) reported that primiparous bovine cows showed a predominance of plateau phase, 2,61 min (44,2%), respect to a decreasing phase of 2,44 min (41,3%) and an increasing phase of 0,86 min (14,5%).
The length of the increasing phase could be explained by the difficulty inherent to the response at prestimulation, to the lower cisternal fraction and to the teat length. On the contrary, the different distribution of milk in the quarters (as in the presence of mastitis in one or more quarters) justifies a long increasing phase and a shorter plateau phase. The predominance of increasing and decreasing phases, have the blind milking of one or more quarters as consequence, with the risk of mastitis onset *(Boselli et al., 2004)*. These hypothesis is confirmed by the correlations between: decreasing phase and somatic cells (r=0,26; P=0,01); plateau phase and somatic cells (r=-0,23; P=0,01).

CONCLUSIONS

It have been observed that the delayed milk yield and the consequent blind milking represent for the primiparous buffalo cows an important risk in mastitis onset. Even if milking time are higher than bovine, it is particularly important to stimulate the udder in premilking or to give feedingstuffs to buffaloes in milking room

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Table 1. Distribution of main relieved parameters.

Variables	Morning(104)	Evening (104)	Total (208)
Milk yield (kg)	4,63±1,98 ***	3,52±1,64 ***	4,08±1,90
Maximum flow (kg/min)	1,57±0,59 ***	1,30±0,56 ***	1,43±0,59
Yield at first 3 min (kg)	3,16±1,48 **	2,58±1,22 **	2,87±1,38
Principal milking time (min)	4,30±2,11 **	3,54±1,84 **	3,92±2,01
Average milk flow in principal milking (kg/min)	0,99±0,39 **	0,85±0,34 **	0,92±0,37
Yield in principal milking (kg)	4,07±2,10***	3,04±1,71***	3,55±1,97
Increasing phase (min)	2,57±2,17	2,79±1,84	2,68±2,01
Plateau phase (min)	1,59±1,15	1,45±1,04	1,52±1,09
Decreasing phase (min)	2,04±1,64	1,68±1,29	1,86±1,49
Total milking time (min)	6,20±3,50	5,92±2,75	6,06±3,14
Somatic cell count* 1.000 (cell./ml.)	245±335 *	367±405 *	315±380

*Different significant levels * (P=0,05), ** (P=0,01), *** (P=0,001).*

Table 2. Main milk flow phases.

Milk flow phases	Morning(104)	Evening (104)	Total (208)
Increasing phase (min)	2,57 (41,5%)	2,79 (47,1%)	2,68 (44,2%)
Plateau phase (min)	1,59 (25,6%)	1,45 (24,5%)	1,52 (25,1%)
Decreasing phase (min)	2,04 (32,9%)	1,68 (28,4%)	1,86 (30,7%)
Total milking time (min)	6,20 (100,0%)	5,92 (100,0%)	6,06 (100,0%)

(Thomas, 2004b). It is a critical point in milking of buffalo, especially in primiparous. Solving these problems, the quantity and quality of productions will be improved.

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9TH BUFFALO WORLD CONGRESS

ARGENTINA 2010

9th WORLD BUFFALO CONGRESS

"The Buffalo in its promised land: America"

Hotel Panamericano Buenos Aires, Argentina

Sunday 25th - Wednesday 28th April, 2010

*** BUFALO POST TOUR: 29th – 5th May, 2010 ***

Organizers

BÜFALO
Argentine Association of Buffalo Breeders

International Buffalo Federation

www.bufalos.org.ar

9TH WORLD BUFFALO CONGRESS

AUTHORITIES

* Explanation: All the names, institutions and organisms mentioned below are to be confirmed.

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Mr. Zoot. Federico Romero

President of the 9th World Buffalo Congress:
Mr. Zoot. Federico Romero

ORGANIZERS
International Buffalo Federation
Argentine Association of Buffalo Breeders

UNDER THE AUSPICES OF
Department of Agriculture, Stockbreeding, Fish and Nutrition (SAGPyA)
National Institute of Farming Technology (INTA)
Lactobacillus Reference Center (Research, Science and Technology National Council)
(CERELA-CONICET)
Government of Corrientes
Government of Formosa
Government of Chaco
ABUAR (Association of Buffalo Breeders of Corrientes)
AFCB (Association of Buffalo Breeders of Formosa)
Rural Society of Formosa
Rural Society of Chaco
National University of Northwestern (UNNE)
National University of Formosa (UNF)
National University of Tucuman (UNT)
University of Buenos Aires (UBA)
Catholic University of Argentina (UCA)
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Dr. Atilio Atencio (Lisandro Alvarado West Center University, Venezuela)
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Dr. Angelo Coletta (Italian National Buffalo Breeders Association-ANASB- Italy)
Dr. Eduardo Bastianetto (Minas Gerais Federal University, Brazil).
Dr Alexandre Rosseto Garcia (EMBRAPA, Belém, Pará, Brasil)

PROVISIONAL CONGRESS SCHEDULE

* Explanation: All the places and people names mentioned below are to be confirmed.
DAY 0 (Sunday 25th April)

Reception: Hotel Panamericano Crowne Plaza Buenos Aires.
Registration: Opening Hour 03.00 pm
Welcome Cocktail: 08.00 pm

DAY 1 (Monday 26th April)

07.30 am – 12.00 pm: Registration
08.30 am – 10.45 am: Opening Ceremony
10.45 am – 11.00 am: Coffee Break (Posters Session and Exhibition)
11.00 pm – 12.00 pm: Plenary Session I (1 hour)
12.00 pm – 01.00 pm: Lunch (to be confirmed) / Exhibition
01.00 pm – 03.00 pm: 2 Parallel Symposium (each one of 2 hours)
03.00 pm – 03.30 pm: Coffee Break (Posters Session and Exhibition)

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03.30 pm – 05.30 pm: 2 Parallel Symposium (each one of 2 hours)
06.00 pm – 08.00 pm: Possible preparatory meeting of the IBF (International Buffalo Federation) Executive Council
08.30 pm: Buenos Aires & Tango Tour
All day: Accompanying Tours

DAY 2 (Tuesday 27th April)

08.00 am – 10.00 am: Plenary Session II (A) (2 hours)
10.00 am –10.30 am: Exhibition and Poster Session (Coffee)
10.30 am – 12.30 pm: Plenary Session II (B) (2 hours)
12.30 pm – 01.30 pm: Lunch (to be confirmed) / Exhibition
01.30 pm – 03.30 pm: 2 Parallel Symposium (each one of 2 hours)
03.30 pm – 04.00 pm: Coffee Break (Posters Session and Exhibition)
04.00 pm – 06.00 pm: 2 Parallel Symposium (each one of 2 hours)
06.00 pm – 08.00 pm: Meeting with the IBF Executive Council
08.30 pm: Farewell Show Dinner
All day: Accompanying Tours

DAY 3 (Wednesday 28th April)

08.00 am – 10.00 am: Plenary Session III (2 hours)
10.00 am – 11.00 am: Coffee Break (Exhibition and Posters Session)
11.00 am – 12.30 pm: 2 Parallel Symposium (each one of 1hour and a half)
12.30 pm – 02.00 pm: Closing Ceremony
Free afternoon (possible visit to La Salamandra Dairy Farm, in Luján, Buenos Aires Province, or visit to the Exhibition)
09.00 pm: Boarding in omnibus suite to the Post Congress Buffalo Tour

DAY 4 (Thursday 29th April)

08.00 am: Visit (lunch included) to Santa Rosa Farm and San Antonio de Salentein Argentina B.V. Farm, in Esquina, Corrientes.
08.00 pm: Arrival at Resistencia and/or Corrientes Hotel.

DAY 5 (Friday 30th April)

08.30 am: Meeting with authorities of the government of Corrientes and Northwestern University (UNNE, Corrientes).
It will be a Plenary Session with a speech of a Keynote Speaker and an authority of UNNE.
12.00 pm: Lunch in "Rincón del Madregón" Farm, from Jorge Félix Gómez, in Empedrado, Corrientes.
02.00 pm: Visit to "Rincón del Madregón" Farm.
06.00 pm: Arrival at the cities of Resistencia and/or Corrientes. Rest and/or Tours around the Cities.

DAY 6 (Saturday 1st May)

09.00 am: Visit to Don Enrique and Don Carlos de Amarilla Agropecuaria S.A. Farms, in Basail, Chaco.
12.00 pm: Lunch in Don Enrique Farm.
05.00 pm: Visit to Rincón del Guayaibí Farm, from the sons of José Pietrantueno, in Mercedes, Corrientes.
10.00 pm: Arrival at the cities of Resistencia and/or Corrientes.

DAY 7 (Sunday 2nd May)

11.00 am: Arrival at the Hotel of Formosa City.
12.00 pm: Lunch with authorities of the Province of Formosa and the University of Formosa.
03.00 pm: Plenary Session with a speech of a Keynote Speaker and the presentation of the Bubalinos Provincial and University Programmes, which are in course. Visit to the Milk Official Control Unit of Bubalinos, in Formosa.

DAY 8 (Monday 3rd May)

08.30 am: Visit to Guazú Cuá and Vidal Cué Farms (lunch included), from Compañía General de Hacienda S.A., in Montelindo, Formosa.
07.00 pm: Arrival at the Formosa City Hotel.
Visit to Santa Úrsula Farm (from Raúl Maglietti), Herradura, Formosa
Visit to Monte Claro Farm (from Ricardo Maglietti), Mariano Boedo, Formosa

DAY 9 (Tuesday 4th May)

08.30 am: Visit to Isla Verde Farm and Dairy Farm, from María Angélica Sbardella de Maglietti and others, in Misión Lahisí, Formosa.
12.00 pm: Lunch and visit to Santa Úrsula Farm and Dairy Farm, from the sons of Raúl Maglietti
06.00 pm: Return to Buenos Aires or return to Corrientes for the Training Courses regarding Reproduction and/or Milk Elaboration, etc.

DAY 10 (Wednesday 5th May)

09.30 pm: Arrival to Buenos Aires, or beginning the Reproduction Training Course, to be realised during 5 (five) days in the Faculty of Veterinary Science of UNNE (Corrientes), in charge of the Dr. M.V. Gustavo Crudeli, titular teacher of the Chair of Physiology of the Reproduction.

DAY 11 (Thursday 6th May)

For the people that will stay in Buenos Aires, it would be organized some Tours around the Country (Ex: Glaciers of the Patagonia, Iguazu Waterfalls, Salta Province in the North, and Lake of Iberá in Corrientes).

SCIENTIFIC PROGRAMME

* Explanation: All the names mentioned below are to be confirmed.

Day 1 (Monday 26th April)

Plenary Session I: 1 hour
Development and perspective of the buffalo in the different regions from the world: Libertado Cruz (Asia), Antonio Borghese (Europa), Jesús Reggeti (America) y Barry Lemcke (Oceania)

2 Parallel Symposium, each one of 2 hours; from 01.00 pm to 03.00 pm

Symposium A: Reproduction, reproductive physiology and biotechnology of the reproduction:
Pietro Sampaio Baruselli (Brazil), Libertado Cruz (Philippines), Antonio Tonizza de Carvalho (Brazil), Luigi Zicarelli (Italia), V. L. Barile (Italia), G. M. Terzano (Italia), Gustavo Crudeli (Argentina), William Gomes Vale (Brazil), R. Kumar (India), S. G. Hassan (Egypt), S. Nasir Hussain Shah (Pakistan), R. Parnapai (Thailand), B. Gasparrini (Italia), A. Fagiolo (Italia), Ohashi (Brazil), Alexandre Rosseto Garcia (Brasil).

Symposium B: Production Sistems

a) In Asia: Panel integrated for:
India: O. P. Dhanda.
China: Yang Bing Zhuang

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Thailand: Metha Wanapat
Vietnam: Mai Van Sanh
Philippines: Libertado Cruz

b) In the rest of the world: Panel integrated for:
Europa and Meddle East: Antonio Borghese
America: Brazil: Otavio Bernardes
Rest of the America: Ricardo Botero Jaramillo, Jesús Reggeti, Claudia Roldán and Marco Zava
Australia: B. Lemcke

2 Parallel Symposium, each one of 2 hours; from 03.30 pm to 05.30 pm.

Symposium C: Breeds, Genetic, Heredity, Selection, Markers

Kamlesh Trivedi (India), Alcides Amorím Ramos (Brazil), Humberto Tonhati (Brazil), Jonas Camargo de Assumpcao (Brazil), Eduardo Bastianetto (Brazil), Antonio Borghese (Italia), Angelo Coletta (Italia), O. P. Dhonda (India), Talat Naseer Pasha (Pakistan), Carlos Taboada Candioti (Argentina), Fernando Sanint Jaramillo (Colombia), Libertado Cruz (Philippines), M.A. Kham (Pakistan), Emmanuela Parlato (Italia), Giuseppe Campanile (Italia)

Symposium D: Precise management of meat rounds up. Rules of management:
Dante Bizzotto (Argentina), Javier Bejarano (Argentina), Nazareno García (Argentina), Erizolei Oliveira da Silva (Brazil), Savigny Serejo Sauáia (Brazil), William Gomes Vale (Brazil), Sergio Toer (Argentina), Rubén Bruyn (Paraguay), Ricardo Maglietti (Argentina), Getulio Marcantonio (Brazil), Bernardo de Hertelendy (Argentina), Luciano Staiak Barbosa (Brazil), Marco Zava (Argentina).

Day 2 (Tuesday 27 th April)

Sesión Plenaria II (A): 2 horas

Meat production, industrialization and commercialization
Surendra K. Ranjhan (India), André Mendes Jorge (Brazil), Nelson Pineda (Brazil), Carlos Ospina (Colombia), Angelo Coletta (Italia), Getulio Suarez (Brazil), Sergio Jaramillo Botero (Colombia), Gladis Rebak (Argentina), Armando Cadoppi (Argentina), Federico Infascelli (Italia), Federico Romero (Argentina), Tiberio Celeste (Brazil), Gustavo Moglia Dutra (Brazil), Joao Ghaspar de Almeida (Brazil), José Cedrés (Argentina), Bruno Cortese (Italia), Jonas Camargo de Assumpcao (Brazil).

Plenary Session II (B): 2 hours

Milk production, industrialization and commercialization
Raffaele Garofalo (Italia), Vilma Penteado, Giuseppe Campanile (Italia), Franco Consalvo (Italia), Otavio Bernardes (Brazil), Nelson Prado (Brazil), Esteban Bullrich (Argentina), Humberto Tonhati (Brazil), Alberto Couto (Brazil), Nelson Prado (Brazil), Martha Núñez de Kairúz (Argentina), Ezequiel Patiño (Argentina), Celeste Guanziroli (Argentina), Alberto Duhau (Venezuela)..

2 Parallel Symposium, each one of 2 hours; from 01.30 pm to 03.30 pm.

Symposium E: Nutrition and feeding:
Raúl Franzolin (Brazil), Federico Infascelli (Italia), Sukhed Ranjhan (India), Giuseppe Campanile (Italia), Antonio Borghese (Italia), Mudgal (India), Metha Wanapat (Talilandia), Julio César Carrero Pulido (Venezuela), Otavio Bernardes (Brazil), Eduardo Bastianetto (Brazil), Luigi Zicarelli (Italia), Floyd Neckles (Trinity and Tobago), P. Lamkin (Trinity and Tobago).

Symposium F: Health, illness, pathologies:
Paul Nicoletti (EE.UU.), Hugo Didonet Lau (Brazil), Gustavo Crudeli (Argentina), Rafael Rodríguez (Venezuela), I.A. Khan (Pakistán), A. Khan (Pakistan), Antonio Borghese (Italia), Federico Kyburg (Argentina), Oscar Racioppi (Argentina), Eduardo Baroni (Argentina), A. Ahmad (Pakistán), V. Veneziano (Italia), Eduardo Bastianetto (Brazil), J. D. Barbosa Neto (Brazil), J. Q. Silvestre (Philippines), Juan Félix Rodríguez Martínez (Venezuela), José Diomedes Barbosa (Brasil).

2 Parallel Symposiums, each one of 2 hours; from 04.00 pm to 06.00 pm.

Symposium G: Buffalos for work:
Harry Cortés Rodríguez (Colombia), Roberto Herrera (Colombia), Gladys Martínez (Brazil), Walter Galindo (Colombia), N. S. Ramaswamy (India), Pereira (Rondonia, Brazil)
Symposium H:
a) Social and regional developments with the buffalo
Otavio Bernardes (Brazil), Yang Bing Zhuang (China), Libertado Cruz (Philippines), Germán Böttger (Argentina), Julio Gómez (Argentina), L. B. Karki (Nepal), Ibrahim Soliman (Egypt), R. A. Pachauri (India), C. Singh (India), Surendra K. Ranjhan (India).
b) Animal Welfare:
N. S. Ramaswamy (India).

Day 3 (Wednesday 28th April)

Plenary Session III: 2 hours

Precise management of dairy rounds up
Héctor Scannone (Venezuela); Pascuale Rossi (Italia), Ettore Bellelli (Italia), Giuseppe Jemma (Italia), Giuseppe Morese (Italia), Bernardo Maglietti (Argentina), Alfonso Bernal Calderón (Colombia), Jesús Reggeti (Venezuela), Miguel Magrane (Argentina), Alberto Couto (Brazil), Eduardo Daher (Brazil), Nelson Prado (Brazil), Alberto Duhau (Venezuela), Gilmer Mendoza (Venezuela).

The Buffalo Leather
César Miller (Brazil), Marco Zava (Argentina) and Juan Nazar (Argentina).

2 Parallel Symposiums, each one of 1 hour and a half, from 11.00 am to 12.30 pm.

Symposium I:
a) Anathomy
G. Pelagalli (Italia)
b) Phisiology (adaptation to the heat); Corporal Condition
E.R Orskov (Russia), Nelcio Tonizza de Carvalho, A. N. Del Barrio (Philippines)

Symposium J: Economy
Pablo Maldonado Vargas (Argentina), Otavio Bernardes (Brazil), Eduardo Bastianetto (Brazil), G. M. Terzano (Italia), Kent Underwood (EE.UU.), Jorge Ordoñez (Venezuela), Alfonso Bernal Calderón (Colombia), Ricardo Botero Jaramillo (Colombia), Ibrahim Soliman (Egypt).

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REPORT OF THE EUROPEAN REGIONAL WORKSHOP ON

Internet-based communication support for ESCORENA Thematic Knowledge Networks in Agriculture

Institute of Natural Fibres
Pozna, Poland, 10-12 April 2008

Prepared by: Marta Majewska, FAO Consultant, Hungary; Nadia Loumbeva, FAO-HQ, Italy-Consultant Knowledge Exchange and Capacity Building; Michal Deme, FAO REUT, Hungary Information and Knowledge Management Officer; Ryszard Kozlowski, ESCORENA Focal Point Coordinator, Poland and Tomaz Bartol, FAO Consultant, Slovenia.

1. BACKGROUND

1.1. What is ESCORENA?

The European System of Cooperative Research Networks in Agriculture (ESCORENA) is a European initiative to enhance sustainable agricultural development and food security by improving the use of information, communication, and associated technologies. The overall aim is to enable ESCORENA members, its partners and users to exchange opinions, experiences, good practices and resources related to agriculture research, and to ensure that the knowledge created is effectively shared and used in the European region and worldwide.
During the 33 years of its existence, ESCORENA was a pioneer initiative for networking in the region in terms of the unique ways of knowledge and information exchange. Regular meetings of the experts, supported by FAO, strongly contributed to the efficient work and commitment within the Network. Today the ESCORENA Network urgently needs to be enhanced with new tools and technologies, in order to overcome problems with access for the community of experts and participating institutions.

1.2. Current opportunities for the ESCORENA network

The introduction of the new tool including the TYPO3 content management system shall take place at the European Regional Workshop "Internet-based communication support for ESCORENA) Thematic Knowledge Networks in Agriculture" in order to prepare the platform for cooperation with similar initiatives within and outside the region.
The objective of the workshop in Pozna_ in April 2008 was to define achievable goals for the establishment of Internet-based knowledge sharing approaches for the ESCORENA network. The Institute of Natural Fibres declared full help and contribution with its staff and facilities to improve ESCORENA activities.
The workshop was attended by 23 participants from twelve different countries, representing the network coordinators of ten networks under ESCORENA, the network secretariat and focal point, the FAO Headquarter and Regional office for Europe, the Agriculture museum network and the focal points of AgroWeb Ukraine and Poland. A complete list of workshop participants is provided in Annex 1.

2. WORKSHOP PROCEEDINGS

April 10th Thursday

2.1. Introductory welcome

Before starting the opening session participants were welcomed by Mr. Demes, Information and Knowledge Management Officer at FAO/REUT, who addressed the current challenges of ESCORENA and possibilities of knowledge sharing through the internet. After a short introduction of participants, Ms. Loumbeva, Consultant in Knowledge Management and Capacity Building at FAO Headquarters, encouraged network coordinators to express their expectations from the workshop and refer to these throughout the workshop. Subsequently a short film about the Institute of Natural Fibres was shown.
On the opening of the workshop Mr. Kozlowski, Director of the Institute for Natural Fibres and host of the workshop, welcomed the participants and officials from the Ministry of Agriculture and Rural Development of Poland and emphasized the importance of research in natural fibres and the necessity to improve technology transfer. Mr. Ardanowski, President Advisor, welcomed participants on behalf of the president of Poland Lech Kaczynski, and highlighted the importance of science in global agriculture as a base for political decisions aiming at ensuring food safety and energetic needs. Mr. Zalewski, Sub-secretary of the Ministry of Agriculture and Rural Development, acknowledged the speed of information exchange and with this regard the contribution of this meeting to international cooperation in agriculture science. Thereafter Mr. Demes thanked the hosts for their hospitality and the workshop organisation and focused on the role of FAO to create a platform for knowledge exchange. He reminded that during the lifetime of ESCORENA network communication technologies and content management systems have been changing and today open source tools might be implemented in order to increase the visibility of experts and to share experience in the different fields of agriculture.

2.2. Presentation of the ESCORENA network

In his opening presentation Mr. Kozlowski presented the background of the ESCORENA network, its history and objectives. He summarised that ESCORENA stands for the voluntary exchange of persons and technologies, in order to establish close links between European researchers and institutions working on the same subject and to stimulate interaction. ESCORENA seeks further to accelerate the transfer of European technology advances to, and cooperation with, developing countries.

2.3. Presentation on the RAMIRAN network website as a possible example for the other networks

Subsequently Mr. Misselbrook, coordinator of the research Network on Recycling of Agricultural, Municipal and Industrial Residues in Agriculture

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(RAMIRAN), illustrated the web utility of this network. The network contains seven different working groups. He reported on conferences being held every 2 years apart from particular meeting of the working groups. As a common effort a glossary of agriculture terminology related to agricultural and industrial residues has been developed. Statistics for www.ramiran.net provide transparent information on quantity and origin of the webpage's visitors.

2.4. Short presentations of the ESCORENA networks' coordinators

CENTAUR

Mr. Wojciechowski, coordinator of the Veterinary Biotechnology and Epidemiology Network (CENTAUR), reports about 2000 links to their webpage http://centaur.vri.cz/ which is recognised on global scale. Around 90 countries are reached by the existent web information which is provided and managed on voluntary basis mainly by Mr. Hruska and Mr. Wojciechowski. The webpage presence enables also for distant lectures and e-learning on veterinary biotechnology and epidemiology.

PASTURES AND FODDER CROPS NETWORK

The Pastures and Fodder Crops Network represented by the coordinator Mr. Alain Peeters, was created in 1962 by originally four countries. In 1972 the network joined ESCORENA and three sub-networks (working groups) were created. Meetings are organised every year. The network is partly supported by the International Centre for Advanced Mediterranean Agronomic Studies (CIHEAM). While no specific webpage exists, materials of meetings are published as proceedings, including in 'Options méditerranéennes (CIHEAM)', on different institute web pages and on the CIHEAM's internet site.

SHEEP AND GOAT NETWORK

The Sheep and Goat network, represented by Mr. Hichem Ben Salem, has been part of the ESCORENA) network since 1995. Conferences within the network are taking place every two years with the support of CIHEAM. No network webpage exists but network's activities are partly visible through the CIHEAM webpage.

BUFFALO NETWORK

Prof. Borghese, coordinator of the BUFFALO network reported that the network was founded in 1992. The Buffalo network produced a link between different scientists and International organizations.The Buffalo Newsletter is published in 1600 copies and distributed free of charge 2 times per year. It organizes meetings and workshop .to promote information and research. Many congresses were organized by the buffalo network almost every year from 1994 until 2008, and promoted the World Buffalo Congress with the International Buffalo Federation, every 3 years starting from 1985 until the last in Caserta in 2007. It promotes international projects involving development countries as in Turkey in 2002, in Azerbaijian in 2003, in Egypt in 2003, in Iran in 2005, in China in 2007, in Indonesia in 2008. It produced the publication of the book "Buffalo Production and Research", edited by Antonio Borghese and FAO as REU Technical series 67.

COTTON NETWORK

The COTTON network, represented by its coordinator Ms Urania Kechagia, was created in 1990 and has members in various Mediterranean countries and also countries outside Europe. Every 4 years plenary meetings take place. FAO support was provided until the year 2000.

SUNFLOWER NETWORK

Mr Skoric, coordinator of the SUNFLOWER network reports that a big challenge for his network is a generational problem related to science. Many researchers retire while younger researchers prefer to work for private companies. Nonetheless important results are reported from hybrid selection. Information on research and results is provided on the webpage of the International Sunflower Association and Serbian web pages. Financial ways has to be explored in order to support networks activities.

NUT NETWORK

The NUT network, represented by the coordinator Ms. Rovira, receives financial support from CIHEAM, research money from the government and the region and from FAO. It publishes a newsletter and is partly visible through the CIHEAM webpage.

OLIVES NETWORK

The OLIVES network, represented by the coordinator Mr. Caballero was established in 1984 by FAO and the Spanish Institute for Agriculture. It comprises four working groups and face to face meetings took place until 1990. After an inactive phase today the network concentrates on genetics and continues publishing a bulletin on olives in printed form and on the web.

SREN NETWORK

The SREN (Sustainable Rural Environment and Energy) Network is working on the improvement of the rural environment and sustainable energy production but has been inactive during last years. There is interest from Mr. Borivoj Sarapatka from the Bioinstitut in Czech Republic to work within the SREN network on organic farming.

2.5. Presentation on Internet-based knowledge-sharing approaches for web-based knowledge networks

In the afternoon Ms. Loumbeva gave an overview of the nature of knowledge and knowledge management, knowledge management at FAO, FAO Thematic Knowledge Networks and the need for a social context in order for an internet-based knowledge network to work. During her presentation, she asked the ESCORENA networks' coordinators to think about why ESCORENA, as a network, is important to them. She also explained explicit and tacit knowledge in international organisations and other institutions. In addition she gave several examples on social networking and encouraged the participants to define the ESCORENA network's potentials and objectives. She also suggested that the ESCORENA networks' coordinators define an ESCORENA network Term of References (ToR) as part of their participation in the workshop. In other words, it would be good to, collectively and depending on the need, create an ESCORENA network ToR plus, if possible, an ESCORENA network strategic plan based on which requests for funding from interested institutions could be made.

2.6. Financial support for the ESCORENA network

FFurther topics discussed during the afternoon session include the financial support of FAO within ESCORENA.

2.7. Web visibility of the ESCORENA network

Concerning web visibility of ESCORENA and its members, Mr. Demes presented the existing webpage (www.ESCORENA.net) and the entry points of the

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member networks. Additionally Mr. Bartol, programme coordinator of AgroWeb, gave examples on content management at AgroWeb and introduced the AGORA program (Access to Global Online Research in Agriculture at http://www.aginternetwork.org/en/) and AGRIS (International Information System for the Agricultural Sciences and Technologies at http://www.fao.org/agris/). Participants agreed that every thematic network should have an entry point from the common ESCORENA webpage. The web pages should share a similar structure and include basic information on their users.

April 12th Friday

2.8. Strengths, weaknesses, opportunities and constraints of the ESCORENA network

On Friday the meeting discussed strengths and opportunities of the ESCORENA network and how to improve the quality and visibility of ESCORENA) on the web.
In the following Ms. Loumbeva summarised the "purpose checklist", a questionnaire regarding the nature and purpose of the ESCORENA Network which was filled by the network coordinators in advance.

2.9 New Chair of the ESCORENA network

First of all, the CENTAUR network was elected by the present coordinators as a member of the ESCORENA network. The creation of and handing over of responsibilities to a focal point for ESCORENA, represented by the Institute of Natural Fibres in Poznan, and namely by Mr. Kozlowski, Ms. Mackiewicz-Talarczyk and Mr. Barriga Bedoya, was discussed by the network coordinators. ESCORENA members agreed on an administrative and technical support of the umbrella network ESCORENA making the network more dynamic and highlighting synergies between the networks. It was further suggested to establish special email addresses for ESCORENA coordinators, to rotate the focal point's location each 3-4 years on a voluntary basis and to inform the partners, especially CIHEAM about this development.

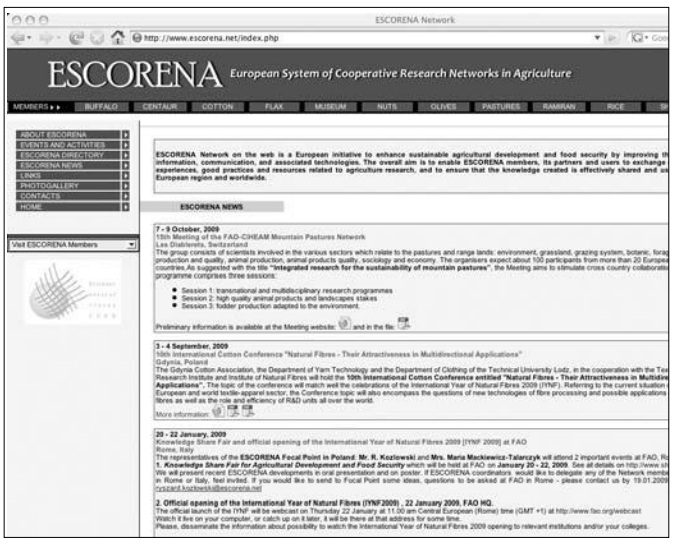
2.10 Suggestions for the ESCORENA webpage design

In the afternoon session concrete suggestions for the ESCORENA webpage design and structure were collected.

3. MEETING OUTCOMES

The following conclusions were drawn during the closing session of the workshop:

- The focal point was voted by all coordinators to be located at the Institute of Natural Fibres in Poznan, Poland. Mr. Kozlowski confirmed the coordination of ESCORENA on voluntary basis;
- The CENTAUR network coordinated by Prof. Hruska and Prof. Wojciechowski became a new member of the ESCORENA network;
- The Agriculture Museum Network represented by Mr. Mackowiak became a new member of the ESCORENA network;
- ESCORENA as an independent network supports network coordinators of various fields of research; it exists and will be sustained through its active members;
- ESCORENA needs to be recognised by other organisations and therefore promoted on existing web pages;
- Much information and materials exist within the networks and can be published on the web;
- The visibility of ESCORENA should be enhanced; Networks coordinators aim for synchronised approaches for information sharing across networks;
- New experts should be selected continuously in order to maintain or enhance the membership;
- Internet based communication cannot replace regular personal meetings;
- Some FAO support can be provided through the Regional office location and its premises in Budapest; the BUFFALO network expressed its interest to organise a symposium in July 12th, 2008 in the premises of the FAO;
- The ESCORENA network plays a useful role for universities, research centres and farmer's advisory services.
- **Develop** new ways of presenting ESCORENA to possible donors focusing on multidisciplinary scientific and innovative cooperation; collectively and depending on the need, **create** an ESCORENA network strategic plan based on which requests for funding from interested institutions could be made
- **Investigate possibilities** of the European program COST Action for ESCORENA activities support
- **Promote** ESCORENA on existing network websites and through FAO; **Inform** FAO Regional and Sub-regional offices about the ESCORENA network activities in order to avoid creation of overlapping thematic networks
- **Explore** means of support from the European Commission; improve project planning
- **Support** capacity building on project proposals and fundraising; explore synergies and common topics among thematic networks
- **Contact and consult** CIHEAM and explain new ESCORENA projects especially about the development of the internet site in order to make sure that the visibility and the support of CIHEAM is sufficiently highlighted
- **Provide** a contact person on web issues from each network who support the focal point in updating the websites
- **Promote and improve** information literacy and the use of information databases through ESCORENA, Link to e-agriculture (http://www.e-agriculture.org/), AGORA (http://www.aginternetwork.org/en/) and AGRIS (http://www.fao.org/agris/).)
- **Facilitate** effective international cooperation through the network
- **Examine** the activities of each of the member networks of the network and identify possible ways of collaboration among these networks



International Buffalo Experts Met with Buffalo Breeders

Buffalo breeders from various European countries and international buffalo experts met on the 10th of October at the 7th German Buffalo Day in Chursdorf / Penig in Saxony. Over the past years the German Buffalo Day has become an important platform for the exchange of latest research results and practical experiences on buffalo production. The event was organised by the Saxon Buffalo Breeding Association and chaired by Mr. Thiele, Head of the German Buffalo Association and German representative of the International Buffalo Federation.
The first presentation of the day focussed on the buffalo population in Bulgaria. Prof. Peeva of the Agricultural Institute in Shumen / Bulgaria gave a review of the historical development of water buffalo keeping in Bulgaria. She also described the formation of the Bulgarian Murrah breed and then went on to speak about the consequences of genetical restrictions when breeding within small populations, which is the case in the majority of the European buffalo populations. In the light of this Prof. Peeva pointed out the needs and prospects for international cooperation in buffalo breeding. The picture of the historical development of the buffalo population in Bulgaria given by Prof. Peeva was complemented with information about the history of buffalo breeding in the Donau-Carpate-Region by a presentation given by Mr. Schobel from Würzburg.
Prof. Dr. Vale from the Federal Rural University of the Amazone region in Belem gave an insight into the management systems for buffalo in Latin America focussing on buffalo production in the Brazilian Amazon Region . In a second presentation he spoke about the occurrence of genetically caused diseases in buffalo.
Prof. Dr. Borghese, as general Secretary of International Buffalo Federation underlined the importance of this meeting and of the reality of the German buffalo livestock in climatic conditions very different from the original hot and humid ones of the buffalo species, anyway with good results and quality products.
Dr. Golze from the Saxon Regional Office for Environment, Agriculture and Geology, who has scientifically accompanied the progress of Saxon buffalo production for several years, presented the results of a study regarding the meat quality of buffalo. In contrast to other European countries where buffalos are kept for their milk, the buffalo in Germany is used as a dual-purpose domestic animal for meat and milk. Most of the animals are kept in a semi-intensive system for meat production. As the production of high-quality meat is the main source of income from buffalo keeping parameters of growth and meat quality are monitored closely.

A recent study conducted by the Saxon Regional Office for Environment, Agriculture and Geology required male and female young buffalos to be slaughtered at an age of 647 days (561 to 757 days, n = 12). Weight at slaughter was 549 kg on average. The dressing percentage was 56.7%, whereas the weight of the two halves was 307.1 kg. The percentage of valuable parts was found to be 62.5% on average. The meat taken off the M. longissimus dorsi contained 21.4% raw protein, 2.5% raw fat, 75.0% water and 1.1% ash. At 48 hours after slaughter pH was at 5.5. The drip loss was 3.5%, loss after grilling 32.6% and loss after cooking 47.3%. Shrinkage on chilling was 3.6% after 14 days and 4.2% after 21 days. According to Minolta CR300 the meat colour was 33.3. Tenderness was measured 5.2 kg at 48 hours post mortem, 3.4 kg after 14 days and 2.8 kg after 21 days.
Research on specific structural characteristics of buffalo milk was the topic of a presentation given by Dr. Schafberg of Martin-Luther-University Halle-Wittenberg.

A special highlight of the Buffalo Day was the presentation and evaluation of buffalo breeding stock which gave an overview of the genetical resources of the German buffalo population. Twenty, predominantly young, animals bred on farms in Saxony and North Germany were evaluated by prof. Peeva, Vale and Borghese.

A. Guglielmetti and M. Golze

THE 3RD NATIONAL BUFFALO CONGRESS IN INDONESIA Tana Toraja, South Sulawesi, 24-26 October 2008

Chalid Talib and Tati Herawati

Indonesian Center of Animal Research and Development
BOGOR, JAVA

INTRODUCTION

Buffalo have an importan role for the farmers especially for poor farmers in Indonesia. Same with the other Asian countries, Buffaloes are used as calves producers, fertilizers, power and meat producers, beside as a livestock for a sources of money in the need of the cash coming. Population of buffalo in the world is around 172.6 million, where 167.5 million in Asia and 2.3 million in Indonesia. The population trend decreases in eastern but increases in the westhern part of Indonesia. This livestock contributes about 46.000 ton of meat or 2.12% of the Indonesia meat consumption. In the last 3 years Indonesia made a special program to fulfill his need of cattle meat consumption, and as buffalo meat in Indonesian market is known as beef, so the buffalo gives a significant potential contribution as about 18% to support beef meat production in Indonesia.

PROGRAM OF THE MEETING

Indonesian Buffalo National Congress, hold annually, started in Sumbawa, West Nusa Tenggara in year 2005. The third meeting in Tana Toraja had the theme of increase the contribution of buffalo meat for fulfilling Indonesian beef consumption, and had been focussed for developing national action programs especially to improve the productivity and to increase the population of buffalo in Indonesia.
More than 100 people attended in the meeting such as Director and Secretary of Directorate General of Livestock Services (DGLS) Prof. Dr. Tjeppy D Sudjana and Prof. Dr. Syamsul Bachry and their staffs, the General Secretary of International Buffalo Federation Prof. Dr. Antonio Borghese, Head of Local Governement in Tana Toraja and Brebes together with their staff, Acting Director of Indonesian Center of Animal Research and Development (ICARD) Dr. Chalid Talib and their scientists, Director of Buffalo Breeding Station Ir. Nusantara MSc and their staff, Scientists from faculty of Animal Husbandry in Indonesia and Indonesian Institute of Science (LIPI), Head of Livestock Services Offices (Disnak/Dinas Peternakan) and their executives from many local governments in Indonesia, stakeholders and buffalo farmers groups.
In the meeting, every head of Disnaks offered presentations about the development of the buffalo action planes in their regions such as population dynamics, development of buffalo farmers organisation, marketing of buffalo, problems and their solutions and their planes for the next years. The scientists invited speakers make their presentation about National Program and Policies for buffalo in Indonesia, an availablity of applied technologies to support buffalo development, some success stories of development models for rearing buffalo, other success models in cattle development that could be applied for buffalo and optimized as local resources for buffalo feeds. The meeting also gave a chance for discussing buffalo development programs for some certain areas that have special problems and for giving solutions. Tana Toraja asked the congress to create a specific program for developing black and white/spotted buffalo that have special high-price in Tana Toraja for burial ceremony of death people.
Prof. Borghese presented as invited speaker the lecture on “The main factors influencing buffalo development”. He underlined the goals and the methodologies to realize genetic and products improvement with a new pilot programme in Indonesia.
Some points are highlighted in the discussion: low of reproduction rate such as the low of calving rate in the buffalo population, late age at the first calving, long calving interval and late of weaning calves. The lengthen of the anoestrus periode is often caused by follicular atresia. All of these problems may have a high relationship with the lake of buffalo feed. Therefore the solution is to enhance buffalo feedingstuffs based on local resources and to alter management in rearing dairy buffaloes and calves, to improve the applied breeding with an open nucleus breeding scheme. The high prices and high demand of black and white buffalo and swamp buffalo in Tana Toraja, so

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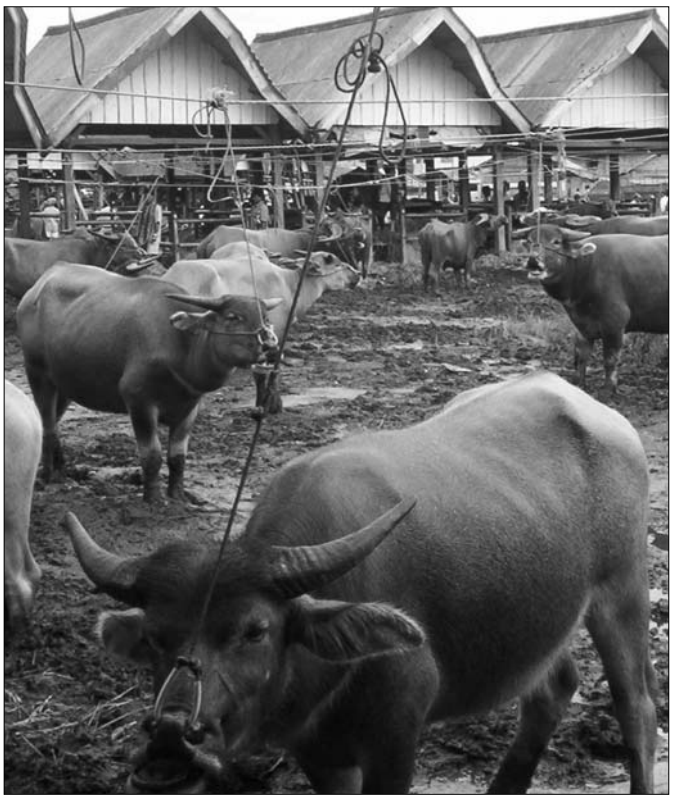
the special program for increasing the number of black and white buffalo are needed soon in the Tana Toraja, while now other places that have surplus could export their buffaloes to Tana Toraja.

There are many kind of buffalo breeds in Indonesia such as black and white buffalo in Tana Toraja, Anoa (wild buffalo) in South Sulawesi, Moa buffalo in Maluku, swim buffalo in the wet lands of Sumatera and Kalimantan, long horn buffalo in Tana Toraja and Sumba-East Nusa Tenggara and Mezam buffalo in Jambi; all of them are swamp buffalo (Bubalus bubalis) except Anoa that is another species (Bubalus depressicornis). Indonesia also had some of dairy buffalo in North Sumatera. The congress suggested that local governments should also put a special attention to select some buffaloes to increase their productivity and genetic improvement and simultaneously to conservate the buffalo genetic resources typical for each region.

In the last day the congress selected the next year meeting place that will be Brebes in Central Java.



THE BUFFALO REALITY IN MACEDONIA

By Antonio Borghese

I was invited by Neshad Azemovski, General Manager of Biosfera Bitola, Centre for education, environment and nature protection, to discuss about status and possibilities for saving buffalo in Macedonia from extinction.
During my visit I had been convinced that the buffalo farms in Macedonia are very few, 4 or 5 at all perhaps, and the total population is very reduced, probably less than 100 animals, but nobody from many people that I met, knows exactly the reality.
After my arriving in Skopje airport on November 10, 2008, I was carried by Neshad and Nikolce Nikolovski in Bitola.
The day after, November 11, I was taken in Debreshte village (near Ropotovo), where I could visit a farm with some local dairy cows and 12 buffaloes *(Fig. 1,2,3)* of Mediterranean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breed, but small and compact, that were bred on natural pasture. The first problem of the farmer was that they have no male and very high consanguinity. The proposed solution is the introduction of artificial insemination by Italian semen to increase the milk production, actually very low, and to introduce different and better genetic basis.

The day after, November 12, I was carried in Mojanci village (near Kocani), where I visited a family farm with 8 buffaloes in the farmyard close their house (Fig 4,5), of the same

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Mediterranean breed , but the animals were bigger than in the previous farm. The farmer produced simple cheese *(Fig.6)* that were sold in local market.
The last day, November 13, I had two meetings in Skopje: the first in the Ministry of Agriculture, Forestry and Water economy, the second in the Ss Cyril and Methodius University with many scientists prof. Sreten Andonov, prof. Gjoko Bunevski, prof. Sonja Srbinovska, dr. Nikola Pacinovski: the conclusion was that a programme to save and develop buffaloes in Macedonia is a priority to maintain biodiversity, to conserve buffalo genetic, that was introduced 5 centuries ago with Turkish invasion, to develop animal farms and typical products for local market and as a basis for tourist economy. The project presented by the Animal Science Institute to the Agricultural Ministry, will be effected with the cooperation of Italy.
The steps of the programme have to be:
1, the knowledge of the situation of buffaloes in Macedonia
2, the import of Italian semen and animals to increase variability and genetic value
3, a training course on reproduction and management
4, the import of technologies
5, the creation of the Macedonia Association of buffalo protectors linked with the I.B.F.


6

"Buffalo Newsletter"
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