

## **Biotechnology Reproduction and Biodiversity Indicators in Buffalo Breeding**

**Iudith Ipate<sup>1</sup>, Florin Banateanu<sup>1</sup>, Cristinel Sonea<sup>1</sup>, Alexandru T. Bogdan<sup>1</sup>, Cornelia Purcarea<sup>2</sup>, Adriana Petrus<sup>2</sup>, Monica Parvu<sup>3</sup>, Simona Ivana<sup>4</sup>, G.F.Toba<sup>1</sup>**

<sup>1</sup>*Romanian Academy, INCE, Center for Studies and Researches Agro forestry Biodiversity, 050711, Bucharest, Calea 13 Septembrie nr.13, Romania*

<sup>2</sup>*Vasile Goldis-Western University of Arad, Bd. Revolutiei, no.94-96, Romania*

<sup>3</sup>*Faculty of the Veterinary Medicine, Spiru Haret University, Bucharest, Romania*

<sup>4</sup>*Faculty of the Veterinary Medicine, USAVM, 050557, Bucharest, Splaiul Independentei 105, Romania*

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**Abstract** Biodiversity is the basic indicator, which expresses the durability and stability zone in direct relationship with the environment and life. Biodiversity monitoring both quantitatively and qualitatively puts us in contact with reality environment and its stability, because biodiversity is constantly changing. Only by measuring the biodiversity we can perceive the sudden changes, which influence directly our life in order to interfere with redesigning the media by ecological reconstruction. The application of biotechnologies to buffalo breeding is paramount to remedy the existing situation. The most numerous Romanian buffalos are the Carpathian type, with valuable gene resource, well adapted to the cold climate for buffalo. Results of artificial insemination in this species are influenced by the quality of semen used. We know that there are periods in which sperm are immobile, so it is important to test the ability of sperm fertilized. This test can be performed in vitro using direct or indirect methods. Utilization of Polamski scale principle remains present, but can be improve and combine with Milovanov resistance and “in vitro” capacitating methods. For determination of fertilizing power we use “in vitro” capacitation methods: direct method, indirect method and “swim-up” technique who are obligatory in E.U. We use this tests because Polamski scale can't be applied, knowing that an immobility of buffalo semen in some conditions is a characteristic of this semen, semen that can be recovery on the normal parameters. Milovanov resistance test have restricted efficiency because NaCl solution have a negative effect over the sperms. Semen charges testing with these new methods they present a good fertilizing power. This test is easier to realize and takes of decision is faster.

**Keywords:** biotechnology reproduction, fertilizing power, biodiversity.

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### **1. Introduction**

These biodiversity erosions have been built using production systems, mechanization, introduction of exotic breed policies inadequate husbandry, diseases, disasters. It was a warning shot, because in the past six years have gone 62 livestock animal species, one species each month, and if it continues at this rate will result in a serious situation in the world. Biodiversity is part of the biodiversity, referring only to the living

component of the ecosystem. Among the work related to indicators of paramount importance to consider the classification of species-indicators, biodiversity indicators back in agricultural landscapes and indicators for assessing large areas, evaluation supraspecific tax-level indicators. Analysis of biodiversity (including assessment of its dynamics) is based on the species.

Internationally, Pakistan region - India is the most popular dairy breeds, Nili-Ravi and Murach being appreciated in terms of scientific research. Thus, Pakistan buffalo milk production accounts for 70% of total milk and meat production 50%.

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\* Corresponding author: Ipate Iudith, Tel.: 0213195130, E-mail: ipate.iudith@gmail.com

Annual growth rate of species was 4.7%, much higher during the last decade. Buffalo milk is preferred in these areas because the percentage of fat is much higher (8%) compared with the cow (3.5%). Buffalos are the animals that use feed more efficiently and therefore inferior quality are grown in areas with diverse climatic conditions, occupying a special place in countries like Thailand, Egypt, Korea, China, Australia, Pakistan, India. Romania is one of the few European countries that still have a significant effect of buffalo (200.000), so research should occupy a special place in this area [1]. The most numerous Romanian buffalos are the Carpathian type, with valuable gene resource, well adapted to the cold climate for buffalo. External characteristics: coat color is unicoloured in back and skin color is also black. Back line is sinuous, i.e. loin and tail point come lower than wither and crump height. In adult animals (males/females) the wither heights are 140-142 / 131-133 cm. Live weights of adult animals (males/females) are 650-680 / 530-560 kg. Main uses are milk, meat and draught power. Milk yield varies according to season of calving, lactations number, level of feeding, and length of lactation. Lactation milk yield (kg) is between 958-1455 kg. The number of days lactation per year is 252-285; age at first calving is 38-42 months; average lactations number is 6-9; age at slaughter for young stock is 22-24 and 350-400 kg or at 3-4 months and 60-100 kg for males and the females are slaughtered at 500-600 kg, usually after the first lactation or after the productive life. In 2003 the female that was included in herd book had 1831 kg milk, with 136 kg fat (7.48%) and 71 kg (3.93%) protein. Age at first calving was 38 month and 6 days and calving interval of these female was 456 days Research on this species is represented in particular by introducing local herd improvement artificial insemination and embryo transfer and management of nutrition, breeding and exploitation of this species [2]. Internationally, biotechnology applied to breeding buffaloes is still at the beginning, is special because of the dilution test analysis related to the season of semen. The work plan is designed as to reinforce the understanding of basic physiological aspects of the buffalo species aimed at improving the reproductive success, especially when considering the application of newly developed reproductive technologies [3]. The main scientific task of the

work plan is the optimization of AI breeding strategies Buffalo sperm is more resistant to shock than bull sperm, but the suitability of freezing and problems with mobility, power and survival times' fertilized frozen semen are still in the research.

## **2. Materials and methods**

In the absence of direct measures at the genetic level, population status and race provides the best indication of trends in diversity [4]. The indicator is made based on official data submitted by the specialized authorities in the project area .

As a method for determining regional buffalo biodiversity were used:

- Cross-sectional systematic study methods, aimed issues, phenomena and processes at a time.

- Cross-sectional studies have given us an overview of the situation in domestic and wild animal populations in the county Hateg being primarily descriptive, but it brought us a large amount of quantitative information, which were processed to obtain study synthetic overall;

- Systematic study of longitudinal methods, which have followed the processes, issues over time (evolution of no. the buffalo)

- The number of units taken so has used both methods of statistical methods and casuistry (case study, monograph, etc.).

- Data collection method was particularly quantitative, because it is an objective method, deductive and generalized. These quantitative approaches have been made in the causal methods.

- Correlation in our case studies has established the link between environmental risk factor, human actions, and ecosystem stability.

Were used as sequential methods, where each method (quantitative or qualitative) research has been addressed in the same turn (see the state of biodiversity of species and breeds of domestic animals) and theoretical and methodological triangulation method for determining specific indicators of biodiversity.

- Have used qualitative research methods to achieve a correlation between the description-classification-connection.

The other research team used modern tools to monitor buffalo livestock biodiversity - molecular tests based on identification, amplification and characterization of nucleic acids, which have revolutionized animal genetic resources conservation for gene assisted selection,

diagnostic pathology and food traceability. Typi original method Fixed - internationally patented Prof. Brem - was first used by researchers in Romania CSCBA to find DNA polymorphism and its applicability in the identification and traceability of meat species biodiversity. The number is slightly decreasing: In 1994 the number of breeding female/male used for AI was 11800 (1,628 in herd book)/ 5 bulls. In 1996 the number of total buffalo was 209,432 that were raised in 86,480 herds; the number of adult females was 97,320 and the number of adult males 2510. In 2003 the number of female in herd book was 275. Type of management is stationary, housing is over 6-8 months / year and feeding is mixes. In most smallholders buffalos are maintained tied in closed barns in winter, while in warm seasons a combination between stable housing with grazing is practiced. The young buffaloes are kept for grazing on pasture. When pastured together, cattle and buffaloes coexist satisfactorily. For testing the buffalo semen quality we use the in vitro methods (direct an indirect technique). Sperms moving is important because reflecting the metabolic status and metabolic type of that's.

-*Direct technique* - use of us, consist in introducing of thawing semen, with viability and motility parameters initially established

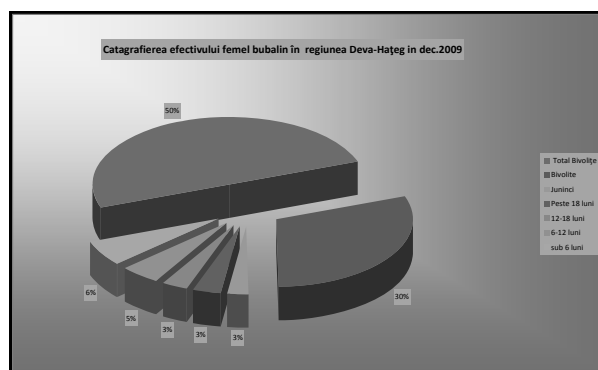
- *Indirect method* - is founding on density gradient (discontinuous) Percoll.

“Swim-up” technique - consisting in balancing 150 µl semen in environment with : Tyrode, albumin, lactate, pyruvate and cultured for one hour in 39 °C, after that, the supernatant rebalancing again in 3 ml TALP and the smear is reading on microscope.

### 3. Results and discussion

Vulnerability of farm animal breeds is caused by the lack of special interest breeders for one breed. Livestock biodiversity specific indicators were developed by this study of genetic diversity d humanity as FAO specific indicators: domestic animal genetic diversity of land was developed for area-specific indicators in buffalo (Figure1).

Determination of genetic diversity index terrestrial domestic animals reveals that the relationship between avian species genetic resources is detrimental to domestic mammalian species.



**Figure.1** Structure female buffalo herd in the county Hateg

Because the number of buffalo declined drastically in this area is necessary to set short-medium and long term strategies.

#### Short Term Strategies

Support and popularize the traditional activities of biodiversity and environment-friendly by extending agricultural entrepreneurship with the principles of biodiversity in farm production to enhance sustainable production.

-Implementation of environmental and development of traditional agriculture marketing of traditional products by creating new organic brands in support of agricultural products from local and regional origin.

#### Medium-term strategy

-Linking biodiversity with demographic pressure in the structure determination of synthetic processing of livestock and LU as the coefficients set by EC Regulation nr.1200/2009, the potential of regional agricultural.

- Promoting the heritage management of genetic resources and preservation of animal and plant that support and factor that determines the stability and proper functioning of the national economy.

#### Long Term Strategies

- Protect, conserve and restore biodiversity through application of technologies conducive to sustainable agriculture;

- Development of research to develop new systems and technologies for sustainable management of genetic resources and their dissemination through training of professional traders and farmers to apply the principles of agricultural biodiversity by accessing funds.

The application of biotechnologies to buffalo breeding is paramount to remedy the existing situation. Expanding biotechnology artificial insemination can be a start in increasing the number of buffalo. Semen testing is mandatory and can be done by direct or indirect laboratory

methods. Submission a brute or freeze semen, after previous processing on the 39°C temperature for a one hour time a go and after that determination of a sperms viability and motility, comparative with pattern parameters on the begin and the end of testing, representing a diagnosis and prediction test for reproductive quality of a bull, semen and fertility of that. Determining forward movements, represent an important parameter chain from donor semen fertility; the calcium influx on the cells inducing mobility type of sperms. On this way, hypermobility appearing like following of massive calcium ions penetration in channels forming after the cholesterol losing in sperms maturation process and can drive on the energetic resources exhaustion of sperm [5].

*-Direct technique* into the vial where they have content 3 ml TALP environment on the 37-38°C temperature, and after a light mixing up with shaking, is separating with centrifugal separator on the 500G in 10 minutes, this operation repeating in maximum three times. After that, the mixture is introducing in thermostat for the one hour and with 39°C temperatures. Parameters reading it were reported on the semen pattern cultured in the thermostat.

*Indirect method* We are using 45 and 90% concentration Percoll solutions in the TALP environment. Thus, pouring out 2 ml solution (45%) on 2 ml solution (90%) Percoll and add 150 µl of semen. All that's, separating on 100G for 10 minutes, and after that, the sediment is remixing in gradient mixture and separated for the 30 minutes on 500G. The sediment is preloaded from 90% gradient and is mixing with TALP for a new separating for 10 minutes on the 200G. Parameters reading were done on the microscope with temperature conditions respecting.

Correlation of sperms percent where swimming progressive is one criterion for diagnosis of semen functionality, but work conditions, especially temperature can become a strong inhibitor factor for mobility [6].

In the cultured time, 10 % from sperm releasing acrosomal contents spontaneous and enzyme concentration is enough for producing a membrane modifiers necessary for others 90%, for this reason, capacitating induction is done with incubation of sperms in big concentration. Sperms with inferior mobility parameter he have a reduce capacity of fecundation, usually with 40%. In the indirect method, "swim-up" and Percoll, the

percent of "live" sperms, with integrity of a viability status and acrosomal membrane, increases over 50-70. The direct test results, indirect with Percoll and "swim-up", as well as mass motility estimation, initial motility, individual motility and forward moving sperms percent evaluation, is effective criteria's in evaluate of buffalo fertilizing power evaluation. This test is easier to realize and takes of decisions is faster than biological prove, artificial insemination of buffalo females and calculating pregnancy percent after ETR diagnosis.

#### **4. Conclusions**

-Biodiversity is the basic indicator, which expresses the durability and stability zone in direct relationship with the environment and life. Biodiversity monitoring both quantitatively and qualitatively puts us in contact with reality environment and its stability, because biodiversity is constantly changing. Only by measuring biodiversity can perceive sudden changes that directly affect our lives to the redesign intervene environment through ecological restoration. - Following the analysis of genetic diversity indicator reconsideration offer high proportion of domestic animals, considering the load of animals per hectare, according to law. That is very important to know the zonal number of animals need to correlate the surface hectare cultivated or pasture. Also be exploited pastures in the area because these areas are not exploited in the existing potential.

-The application of biotechnologies to buffalo breeding is paramount to remedy the existing situation.

-Semen charges testing in this new ways have present a good fertilizing power and is very necessary because of this fact: Polamski scale can't be used on buffalo, cattle and the Milovanov test for resistance have a limit ate efficiency.

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