eISBN: 978-1-68108-417-6
ISBN: 978-1-68108-418-3

THE BUFFALO (*BUBALUS BUBALIS*) PRODUCTION AND RESEARCH

Editor
Giorgio A. Presicce

Bentham e Books

The Buffalo (*Bubalus bubalis*) – Production and Research

Edited by

Giorgio A. Presicce
ARSIAL – Regione Lazio, Rome, Italy

Reproduction and Production of Water Buffaloes (*Bubalus bubalis*) Around the World

Editor: Giorgio A. Presicce

eISBN (Online): 978-1-68108-417-6

ISBN (Print): 978-1-68108-418-3

Published by Bentham Science Publishers – Sharjah, UAE.

First published in 2017.

BENTHAM SCIENCE PUBLISHERS LTD.

End User License Agreement (for non-institutional, personal use)

This is an agreement between you and Bentham Science Publishers Ltd. Please read this License Agreement carefully before using the ebook/echapter/ejournal (**“Work”**). Your use of the Work constitutes your agreement to the terms and conditions set forth in this License Agreement. If you do not agree to these terms and conditions then you should not use the Work.

Bentham Science Publishers agrees to grant you a non-exclusive, non-transferable limited license to use the Work subject to and in accordance with the following terms and conditions. This License Agreement is for non-library, personal use only. For a library / institutional / multi user license in respect of the Work, please contact: permission@benthamscience.org.

Usage Rules:

1. All rights reserved: The Work is the subject of copyright and Bentham Science Publishers either owns the Work (and the copyright in it) or is licensed to distribute the Work. You shall not copy, reproduce, modify, remove, delete, augment, add to, publish, transmit, sell, resell, create derivative works from, or in any way exploit the Work or make the Work available for others to do any of the same, in any form or by any means, in whole or in part, in each case without the prior written permission of Bentham Science Publishers, unless stated otherwise in this License Agreement.
2. You may download a copy of the Work on one occasion to one personal computer (including tablet, laptop, desktop, or other such devices). You may make one back-up copy of the Work to avoid losing it. The following DRM (Digital Rights Management) policy may also be applicable to the Work at Bentham Science Publishers’ election, acting in its sole discretion:

- 25 ‘copy’ commands can be executed every 7 days in respect of the Work. The text selected for copying cannot extend to more than a single page. Each time a text ‘copy’ command is executed, irrespective of whether the text selection is made from within one page or from separate pages, it will be considered as a separate / individual ‘copy’ command.
- 25 pages only from the Work can be printed every 7 days.

3. The unauthorised use or distribution of copyrighted or other proprietary content is illegal and could subject you to liability for substantial money damages. You will be liable for any damage resulting from your misuse of the Work or any violation of this License Agreement, including any infringement by you of copyrights or proprietary rights.

Disclaimer:

Bentham Science Publishers does not guarantee that the information in the Work is error-free, or warrant that it will meet your requirements or that access to the Work will be uninterrupted or error-free. The Work is provided "as is" without warranty of any kind, either express or implied or statutory, including, without limitation, implied warranties of merchantability and fitness for a particular purpose. The entire risk as to the results and performance of the Work is assumed by you. No responsibility is assumed by Bentham Science Publishers, its staff, editors and/or authors for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products instruction,

advertisements or ideas contained in the Work.

Limitation of Liability:

In no event will Bentham Science Publishers, its staff, editors and/or authors, be liable for any damages, including, without limitation, special, incidental and/or consequential damages and/or damages for lost data and/or profits arising out of (whether directly or indirectly) the use or inability to use the Work. The entire liability of Bentham Science Publishers shall be limited to the amount actually paid by you for the Work.

General:

1. Any dispute or claim arising out of or in connection with this License Agreement or the Work (including non-contractual disputes or claims) will be governed by and construed in accordance with the laws of the U.A.E. as applied in the Emirate of Dubai. Each party agrees that the courts of the Emirate of Dubai shall have exclusive jurisdiction to settle any dispute or claim arising out of or in connection with this License Agreement or the Work (including non-contractual disputes or claims).
2. Your rights under this License Agreement will automatically terminate without notice and without the need for a court order if at any point you breach any terms of this License Agreement. In no event will any delay or failure by Bentham Science Publishers in enforcing your compliance with this License Agreement constitute a waiver of any of its rights.
3. You acknowledge that you have read this License Agreement, and agree to be bound by its terms and conditions. To the extent that any other terms and conditions presented on any website of Bentham Science Publishers conflict with, or are inconsistent with, the terms and conditions set out in this License Agreement, you acknowledge that the terms and conditions set out in this License Agreement shall prevail.

Bentham Science Publishers Ltd.
Executive Suite Y - 2
PO Box 7917, Saif Zone
Sharjah, U.A.E.
Email: subscriptions@benthamscience.org

BENTHAM
SCIENCE

CONTENTS

Gianluca Neglia and *Giovanna Bifulco*

Bianca Gasparrini

Gautam Kumar Deb, Rangsun Parnpai and *Il Keun Kong*

FOREWORD

Buffaloes are members of the kingdom Animalia, phylum Chordata, class mammalian, order Artiodactyla and family Bovidae. They are further classified into two main species, the African wild buffalo (*Syncerus*) and the Asian buffalo (*Bubalus*). The Asian buffalo is further classified into the river (*Bubalus bubalis*) and swamp type (*Bubalus carabensis*) species. The study related to their origins indicate that swamp buffaloes may have originated in China and were domesticated about 4,000 years ago, while the river type may have originated from India some 5,000 years ago. Thus, the buffalo has been domesticated more recently as compared to *Bos taurus* and *Bos indicus* cattle, both domesticated ~10,000 years ago.

According to the FAO, the total population of buffaloes in the world during 2013 was 193.8 million. Asia alone accounts for the majority of heads, 187.9 million buffaloes that constitute 96.96 % of the total population. Because of its usefulness, the buffalo has been moved to Africa (4.2 million; 2.17%), America (1.34 million; 0.71%), Europe (0.43 million; 0.22%) and Oceania (210 numbers), and it is becoming popular in many non-buffalo rearing countries of these subcontinents. India possesses the largest number (109.4 million: 56.4%) and most of the best breeds of buffaloes (such as Murrah, Nili-Ravi, Banni, Mehsana, Bhadavari, Jafarabadi, Surti *etc.*).

Buffaloes are very important animals in Asian farming with milk, meat and hides as their major contribution to the zoo-economy, together with other forms of contribution within field-work such as pumping water, ploughing, planting and cultivation of crops, puddling of rice fields, hauling carts to carry various materials and people, thrash grains and crush sugar canes, *etc.* Buffaloes are also used for social and cultural events, sports and religious purposes. It contributes to about 55% of the milk produced in India and about 10% of the total global milk production. Buffalo milk has a high level of nutrients, and many consumers prefer it because of its white color, high fat content and flavor. Similarly, buffalo meat is amazingly tender, juicy with a slightly sweet flavor and it is lower in fat, calories and cholesterol than cattle beef, and higher in protein. The buffalo has an intrinsic ability to efficiently convert poor quality forages and crop residues of marginal areas into high quality milk and meat and it has exceptionally long productive life; in fact a healthy female may have as many as nine to ten lactations. Because of its colour and immense economic value, the buffalo is often called “Black Gold", and today more human beings depend on them than on any other domestic animal.

Depending on the geographical situations and the purpose for which they are used, buffaloes are managed differently all over the world. Slowly buffalo rearing is changing from the backyard to commercial enterprises and is following the path of cattle industry. Buffalo has an excellent potential for milk and meat production, and therefore development and application of simple technologies to overcome deficiencies in breeding, nutrition, healthcare, management and welfare and simultaneously judicious application of current technologies such as genomics, proteomics, reproductive biotechnologies, nanotechnology, bioinformatics *etc.*, may lead to its faster development.

Scientific literature on buffaloes has mushroomed in the last two decades, covering various aspects of buffalo production. In order to maximize productive and reproductive performances, newly developed technologies have been implemented in buffalo farming and management, and in some instances with excellent results. This book has 15 chapters, from the contribution of selected renowned educators / scientists from different buffalo rearing countries, dealing with the most recent advances ranging from reproductive physiology to nutrition, welfare, milk production and genetics. The book is aimed at magnifying the importance of this species in the world, and highlights areas of research that need to be explored urgently.

I am sure this book will be a valuable reference for researchers, policy experts, professionals, and above all, educators as well as under-graduate and post-graduate students interested in the bubaline species.

Prof. AK Misra
Vice-Chancellor
Maharashtra Animal and Fishery Sciences University
Seminary Hills
Nagpur-440 001
Maharashtra, India

PREFACE

Scientific literature on buffaloes (*Bubalus bubalis*) has skyrocketed in the last two decades, ranging from production to reproduction issues. Buffaloes have played an instrumental role in so-called emerging countries of the Asian continent, especially thanks to their intrinsic ability to convert poor quality forages and crop residues of marginal areas, into high quality milk and meat. A special focus of interest has been addressed from researchers to the river subspecies, being the most productive across countries of the European as well as the Asian continents. In fact, in some countries, the river buffalo has shown over the years, an increasing trend in the number of available heads, whereas in others, swamp buffaloes have decreased dramatically. In order to maximize productive and reproductive performances, newly developed technologies have been implemented in buffalo farming and management, and in some instances with excellent results. This book, presenting the most recent advances in buffalo production and research, aims at magnifying the importance of this species in the world and at highlighting areas of research still in need to be more deeply explored.

Giorgio A. Presicce
ARSIAL – Regione Lazio,
Rome,
Italy

List of Contributors

Pietro Sampaio BARUSELLI	Departamento de Reprodução Animal, FMVZ-USP, São Paulo, SP, Brazil
Giovanna BIFULCO	Department of Veterinary Medicine and Animal Production, Federico II University, V. Delpino 1, 80137, Naples, Italy
Antonio BOSCO	Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, CREMOPAR Regione Campania, Italy
Ada BRAGHIERI	Scuola di Scienze Agrarie, Forestali, Alimentari ed Ambientali, Università della Basilicata, *Via* dell'Ateneo Lucano 10, 85100, Potenza, Italy
Narongsak CHAIYABUTR	Dairy Cattle Physiology Division, National Dairy Research, Institute, Karnal-13200, Haryana, India
Vongpasith CHANTHAKHOUN	Department of Animal Science, Faculty of Agriculture and Forest Resources, Souphanouvong University, Laos
Giuseppe CRINGOLI	Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, CREMOPAR Regione Campania, Italy
Libertado C. CRUZ	Philippine Carabao Center (PCC), Science City of Muñoz 3120, Nueva Ecija, Philippines
Gautam Kumar DEB	Division of Applied Life Science (BK21 Program), Graduate School of Gyeongsang National University and Animal Production Research Division, Bangladesh Livestock Research Institute, Savar, Dhaka–1341, Bangladesh
Giuseppe DE ROSA	Dipartimento di Agraria, Università di Napoli Federico II, *Via* Università 133, 80055 Portici (NA), Italy
Bianca GASPARRINI	Department of Veterinary Medicine and Animal Production, Federico II University, *Via* F. Delpino 1, 80137 Naples, Italy
Fernando GRASSO	Dipartimento di Agraria, Università di Napoli Federico II, *Via* Università 133, 80055 Portici (NA), Italy
Alessandra IANNUZZI	National Research Council (CNR), Institute of Animal Production Systems in Mediterranean Environment (ISPAAM), Laboratory of Animal Cytogenetics and Gene Mapping, Naples, Italy
Leopoldo IANNUZZI	National Research Council (CNR), Institute of Animal Production Systems in Mediterranean Environment (ISPAAM), Laboratory of Animal Cytogenetics and Gene Mapping, Naples, Italy

Pichad KHEJORNSART	Tropical Feed Resources Research and Development Center (TROFREC), Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Khon Kaen, 20004, Thailand
Il Keun KONG	Division of Applied Life Science (BK21 Program), Graduate School of Gyeongsang National University and Institute of Agriculture and Life Sciences, Jinju 660-701, Gyeongnam, Republic of Korea
Maria Paola MAURELLI	Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, CREMOPAR Regione Campania, Italy
Claro N. MINGALA	Philippine Carabao Center (PCC), Science City of Muñoz 3120, Nueva Ecija, Philippines
Fabio NAPOLITANO	Scuola di Scienze Agrarie, Forestali, Alimentari ed Ambientali, Università della Basilicata, *Via* dell'Ateneo Lucano 10, 85100, Potenza, Italy
Gianluca NEGLIA	Department of Veterinary Medicine and Animal Production, Federico II University, V. Delpino 1, 80137, Naples, Italy
Egil Robert ORSKOV	International Feed Resource Unit, Macaulay Land Use Research Institute, UK
Corrado PACELLI	Scuola di Scienze Agrarie, Forestali, Alimentari ed Ambientali, Università della Basilicata, Via dell'Ateneo Lucano 10, 85100, Potenza, Italy
Rangsun PARNPAI	Embryo Technology and Stem Cell Research Center, School of Biotechnology, Suranaree University of Technology, Nakhon Ratchasima 30000, Thailand
Alfredo PAUCIULLO	National Research Council (CNR), Institute of Animal Production Systems in Mediterranean Environment (ISPAAM), Laboratory of Animal Cytogenetics and Gene Mapping, Naples, Italy
Ruangyot PILAJUN	Tropical Feed Resources Research and Development Center (TROFREC), Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Khon Kaen, 20004, Thailand
Giorgio Antonio PRESICCE	ARSIAL, Regione Lazio, *Via* R. Lanciani, 38 - 00161, Rome, Italy
Laura RINALDI	Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, CREMOPAR Regione Campania, Italy
Marina SANSINENA	Technical Department of Argentine Buffalo Breeders Association, International Consultant, Arcos 1325, 1426, Buenos Aires, Argentina
Vo Thi Kim THANH	Department of Animal Physiology, Hue University of Agriculture and Forestry, Vietnam

Ramesh C. UPADHYAY	Dairy Cattle Physiology Division, National Dairy Research Institute, Karnal-13200, Haryana, India
Marvin A. VILLANUEVA	Philippine Carabao Center (PCC), Science City of Muñoz 3120, Nueva Ecija, Philippines
M. WANAPAT	Tropical Feed Resources Research and Development Center (TROFREC), Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Khon Kaen, 20004, Thailand
Marco ZAVA	Technical Department of Argentine Buffalo Breeders Association, International Consultant, Arcos 1325, 1426, Buenos Aires, Argentina
Luigi ZICARELLI	Dipartimento di Medicina Veterinaria e Produzioni Animali - Università "Federico II" - Napoli, *Via* Delpino, 1 - 80137 Napoli, Italy

DEDICATION

To my mother, to my father always with me, to my children. To my sources of inspiration.

CHAPTER 1

River and Swamp Buffaloes: History, Distribution and their Characteristics

Claro N. Mingala[*], **Marvin A. Villanueva** and **Libertado C. Cruz**

Philippine Carabao Center, Science City of Muñoz 3120, Nueva Ecija, Philippines

Abstract: Water buffalo, whether it belongs to the swamp or river type, is an important animal resource aside from cattle, whose great potential as source of products of animal origin and as a tool for research has been widely recognized. With a population of about 168 million, buffaloes are widely distributed in many countries around the world, mainly in the Asian continent as an important source of milk, meat, hide and draft power. This paper presents the history, world distribution, breeds, the characteristics of the two types of buffaloes, and the genetic improvement achieved in this species.

Keywords: Breed, Crossbreeding, Draft, Milk, River and swamp buffalo.

1. INTRODUCTION

The water buffalo and the men, who have been raising it with love for centuries, have been closely related and dependent on each other, so the buffalo has acquired a great social and cultural importance to human beings. The buffalo appears in the legends and folk arts of many people, especially the Asian, becoming an inseparable part of human life.

2. HISTORY

The Asian buffalo or the water buffalo (*Bubalus bubalis*) belongs to class Mammalia, sub-class Ungulata, order Artiodactyla, sub-order Ruminantia, family

---

[*] **Corresponding author Claro N. Mingala:** Philippine Carabao Center, Science City of Muñoz 3120, Nueva Ecija, Philippines; Tel: +63 44 4560731; Fax: +63 44 4560730; E-mail: cnmingala@hotmail.com

Giorgio A. Presicce (Ed.)

Bovidae, sub-family Bovinae, and tribe Bovini. Under the tribe Bovini three groups are included, the Bovina (cattle), Bubalina (the Asian buffalo), and Syncerina (the African buffalo). The Asian and the African buffaloes are classified under the genus names *Bubalus* and Syncerus, respectively, which are generally similar despite some anatomic differences between them.

The African buffalo (Syncerina group) includes only one species (*Syncerus caffer*) and some subspecies. The Asian buffalo (*Bubalus*) includes three different buffalo species: Anoa (*Bubalus depressicornis*) from the Island of Celebes, Tamaraw (*Bubalus mindorensis*) from the Island of Mindoro in the Philippines and Arni (*Bubalus arnee*) or the Indian wild buffalo. Of these four species of African and Asian buffaloes only the Indian wild buffalo, Arni has been domesticated and received the species name *bubalis*. The other three types have not been domesticated. The domestic buffalo is presently raised in the world under the name water buffalo and is classified as *Bubalus bubalis* [1].

Information about the origin and domestication period of the Indian wild buffalo is lost back in ancient times, although archeological evidence shows that both Asia and Europe have relied on water buffaloes for a very long time. According to Shalash [2], there is archeological evidence of buffalo domestication dating back to 2,000 BC in Mesopotamia and the valley of Indus. In 1980, Prof. Sieh Chen-Hsia of Nanking Agricultural College, China, however, reported on more recent archeological investigations in China (Chekiang Province) which give grounds to the assumption that the domestication of the buffalo has started 7,000 years ago. On the contrary, Bhat [3] believes that this has happened about 5,000 years ago on the Indian sub-continent, more precisely in the valley of Indus. Their horns, coarse skin, wide muzzles, and low-carried heads have been represented on seals struck since 5,000 years ago in the Indus Valley, suggesting that in India and Pakistan such animals had been already domesticated since that time. Accordingly, the domestication of swamp buffaloes also took place in China independently about 1,000 years later [4].

Water buffalo did spread widely all over Asia and was introduced in parts of Europe, the Near East and Egypt, the Caucasian region of the former USSR and later in South America. Buffaloes were probably unknown to ancient Egyptians, Romans and Greeks and this is possibly the reason why such animals have not been mentioned in their literature or seen in their arts; nevertheless buffaloes were used in China 4,000 years ago. Arabs began moving the buffaloes from Mesopotamia around 600 A.D. to the Near East (today Syria, Israel and Turkey), whereas the same animals were introduced by pilgrims and crusaders from their return from the Holy Land into Europe in the Middle Ages. Buffaloes adapted well to the malaric Pontine marshes characterizing the southeast area of Rome and south of Naples, and established themselves also in other territories today known as Hungary, Romania, Yugoslavia, Greece and Bulgaria, and stayed there ever since. In Egypt, medieval villagers began adopting and using the buffaloes, and have remained since then even in modern Egypt the most important domestic animals, in fact doubling the population up to a million heads in the course of the last 50 years [1, 4].

Since 84 years ago, Brazil invested into buffalo production by importing groups of animals mostly from Italy and India. A similar attitude has been witnessed also in nearby countries like Trinidad by importing buffaloes from India in the early 90s, whereas other countries like Venezuela, Colombia and Guyana have become familiar with buffalo import much more recently. Similarly, some remaining countries of the American continent like Costa Rica, Ecuador, Cayenne, Panama and Suriname began importing small herds of buffaloes in the 70s.

Even in Papua New Guinea, the buffaloes have been imported and the new environment has been fitting the new species very well. Comforted by such good results, in the 60s scientists evaluated buffalo performances in Papua New Guinea and more animals were imported from Australia. As a result, the whole lot of buffaloes introduced have been performing so well that they have out-performed the cattle counterpart both in terms of born calves and meat produced. In fact, buffaloes differently from cattle, as it also happens elsewhere, are able to maintain their physiological functions and appetite, despite the heat and humidity typical of the region. For these reasons the government of Papua New Guinea has since decided to import additional water buffaloes along the years, reaching today a total of almost 3,500 heads.

Buffaloes have not been recognized for their potential for a long time in the

CHAPTER 2

The Cytogenetics of the Water Buffalo

Alessandra Iannuzzi* and **Leopoldo Iannuzzi**

National Research Council (CNR), Institute of Animal Production Systems in Mediterranean Environment (ISPAAM), Laboratory of Animal Cytogenetics and Gene Mapping, Naples, Italy

Abstract: Though world buffalo population is about 1/9 of cattle population, more human beings depend from buffaloes, especially in South-East Asia. Indeed, there are about 168 million water buffaloes in the world, mostly (161 million) raised in Asia. The River buffalo has received great attention from West countries which are particularly interested to both milk and meat production. For this reason, the genetic improvement of buffaloes still remains one of the most important goal in this species. Cytogenetic is one of the biotechnologies which supports the genetic improvement of buffaloes, especially for the selection of reproducers. In this chapter, an update of the latest results obtained in the cytogenetics of buffaloes is reported, starting from its cytotaxonomy and going through clinical and molecular cytogenetics, cytogenetic investigations and breeding objectives.

Keywords: Chromosome abnormality, Cytogenetics, Evolution, Fertility, Water buffalo.

1. INTRODUCTION

Though buffaloes are about 1/9 of cattle, they interest a world human population greater than that raising cattle. For this reason this species attracts the interest of many people, especially in East countries, and is of great economic importance. Among buffaloes, the Asiatic water buffalo, in particular the riverine type, is the most important one. The main findings obtained so far in cytogenetics of this species, are summarized in this chapter.

---

[*] **Corresponding author Alessandra Iannuzzi:** National Research Council (CNR), Institute of Animal Production Systems in Mediterranean Environment (ISPAAM), Laboratory of Animal Cytogenetics and Gene Mapping, Naples, Italy; Tel: +39 081 5964977, +39 081 5966006; Fax: +39 081 5965291; E-mail: alessandra.iannuzzi@ispaam.cnr.it

Giorgio A. Presicce (Ed.)

2. ORIGIN AND EVOLUTION

The buffalo belongs to the Bovini tribe, Bovidae family, Ruminantia suborder and Cetartiodactyla order (Table **1**). The African buffalo (*Syncerus caffer*) and the Asiatic buffalo (*Bubalus bubalis*) are the most important species raised in the world. Within the African buffalo (*Syncerus caffer*) three subspecies can be identified: the Cape Buffalo (*Syncerus caffer caffer*), the Forest Buffalo (*Syncerus caffer nanus*) and the Sudan Buffalo from West Africa (*Syncerus caffer brachyceros*) [1, 2]. Karyotypes of these species differ among them and represent the most simple method to distinguish their genotypes, also in their inter-specific hybrids. Cytogenetic investigations have shown that *Syncerus caffer caffer* has 2n = 52 with a fundamental number (FN) equal to 60, while *Syncerus caffer nanus* has 2n = 54 and FN = 60. Crosses between these two species are possible, with F1-hybrid having 2n = 53. This condition may be cause of reduced fertility for the formation of unbalanced gametes due to erroneous meiotic segregations, as it occurs in other species [3, 4]. Four (*Syncerus caffer caffer)* and three (*Syncerus caffer nanus*) biarmed autosome pairs are the cause of the different diploid number found in these two species, being all remaining chromosomes acrocentric, including the X-chromosome (the largest acrocentric one) and the Y-chromosome. The biarmed pairs in *S.c. caffer* have been originated by Robertsonian translocations of cattle (ancestral bovid) chromosomes 1;13, 2;3, 5;20 and 11;29 [5].

Two main species are considered in the Asiatic buffalo or water buffalo (*Bubalus bubalis*): the river buffalo (2n = 50, FN = 60) and the swamp buffalo (2n = 48, FN = 58). Since all chromosomes (chromosome arms) have been conserved in the two species, crosses between them are possible, although the hybrid has 49 chromosomes and may originate reproductive problems due to unbalanced gametes. The river buffalo (2n = 50) is the most important and numerous buffalo species in the world. It has five submetacentric autosomes, being the remaining chromosomes acrocentric, including the X-chromosome (the largest one) and the Y chromosome. The five submetacentric river buffalo chromosomes (BBU) originated by Robertsonian translocations of cattle homologous (cattle ancestor) chromosomes and relative syntenic groups (U): BBU1 (1;27-U10/U25), BBU2 (2;23-U17/U20), BBU3 (8;19-U18/U21), BBU4 (5;28-U3/U29), and BBU5

(16;29-U1/U7) according to the standard karyotypes of both river buffalo [6] and cattle [7].

The swamp buffalo (2n = 48) karyotype differs from the river type for the presence of a large chromosome (chr 1), which was formed by tandem fusion translocation between river buffalo chromosomes 4 (BBU4) and 9 (BBU9). Since BBU4 was formed by translocation (centric fusion) of cattle homologous chromosomes 5 and 28 [6], and BBU9 is homologous to cattle chromosome 7 [6], three cattle (ancestor) homologous chromosomes (and bovine synthenic groups - U) constitute swamp buffalo chromosome 1: BTA5 (U3), BTA28 (U29) and BTA7 (U22) [8].

By comparing the karyotypes of African (*Syncerus*) and Asiatic (*Bubalus*) buffaloes, it appears evident that no biarmed chromosome pairs are common between the two species [6 - 9].

In Table **1** cytogenetic data of different species of buffaloes in the world are summarized.

Table 1. Cytotaxonomy of buffaloes in the world and their chromosome diploid (2n) and fundamental (FN) number.

Order	Cetartiodactyla				
Suborder	Ruminantia				
Family	Bovidae				
Species	African buffalo (*Syncerus caffer*)			Asiatic buffalo (*Bubalus bubalis*)	
Subspecies	Large black savannah or Cape Buffalo (*Syncerus caffer caffer) 2n=52 NF=60*	Intermediate Sudan buffalo from West Africa (*Syncerus caffer brachyceros) 2n=53 NF=60*	Small reddish forest buffalo (*Syncerus caffer nanus) 2n=54 NF=60*	River buffalo 2n=50 NF=60	Swamp buffalo 2n=48 NF=58

3. CYTOGENETIC INVESTIGATIONS

3.1. G- and R-banding

G(Giemsa) and R-(reverse) banding are the usual techniques applied for the

CHAPTER 3

Molecular Genetics and Selection in Dairy Buffaloes: The Italian Situation

Alfredo Pauciullo[*] and **Leopoldo Iannuzzi**

National Research Council (CNR), Institute of Animal Production Systems in Mediterranean Environment (ISPAAM), Laboratory of Animal Cytogenetics and Gene Mapping, Naples, Italy

Abstract: The Italian river buffalo was characterized by an extensive period of isolation, which did not allow crossbreeding. This has brought to a morpho-functional differentiation of the Mediterranean type, whose population has increased 19 fold in Italy in the past fifty years. This increase is mainly due to the rising interest in the productive characteristics of this rustic animal; actually bred mainly as dairy purpose animal. Marker assisted selection (MAS) might be a promising choice for planning appropriate breeding schemes for Italian river buffaloes. In this respect, the genetic markers significantly associated to milk yield traits may give the right information for the identification of animals with high breeding value. The literature associated with different aspects of the genetic progress in buffalo is abundant, and this chapter is a review of the molecular bases for the improvement of the quali-quantitative characteristics of the Italian dairy buffaloes occurred during the last decade.

Keywords: Casein cluster, Genetic improvement of dairy traits, Milk yield, Molecular selection, River buffalo.

1. INTRODUCTION

The domestic water buffalo was historically split into the swamp and river subspecies, due to their difference in morphology, behaviour, and chromosome number (2n=48 and 2n=50, respectively). Swamp buffalo is predominant in Southeast Asia and China, whereas the river type is mainly found in India,

---

[*] **Corresponding author Alfredo Pauciullo:** National Research Council (CNR), Institute of Animal Production Systems in Mediterranean Environment (ISPAAM), Laboratory of Animal Cytogenetics and Gene Mapping, Naples, Italy; Tel: +39 011 6708598; Fax. +39 011 6708506; E-mail: alfredo.pauciullo@ispaam.cnr.it

Giorgio A. Presicce (Ed.)

Southwest Asia and in the Mediterranean area [1]. Despite their phenotypic differences, there is still a great interest on their time of domestication [2], as well as a debate as to consider appropriate their classification into two related subspecies [3]. In fact, molecular evidences based on mitochondrial DNA analysis [4, 5], molecular markers [6, 7] and Y-chromosome gene variations [8] showed that the two types are distinct and the separation of swamp and river type pre-dates domestication. They share several haplotype both at genomic and mitochondrial level, but the swamp and the river buffaloes constitute two distinct subspecies.

In the last years, several research projects have focused on the buffalo genome. In particular, 8 different consortium groups are working to fill the gap with other livestock species. Five out of 8 projects are related to transcriptome sequencing, 2 focused on the genome sequencing and one is relative to radiation hybrid. The NCBI database (http://www.ncbi.nlm.nih.gov/assembly/GCA_000471725.1/#/st) reports the following state of art for the UMD CASPUR WB 2.0 project updated at 30th September 2013:

UMD CASPUR WB 2.0	
Assembly level:	Scaffold
Genome representation:	Full
Total sequence length	2,836,150,610
Total assembly gap length	74,388,041
Gaps between scaffolds	0
Number of scaffolds	366,982
Scaffold N50	1,412,388
Number of contigs	630,367
Contig N50	21,938
Total number of chromosomes	0

Although the sequencing of buffalo genome is complete, currently the annotation of the sequences is not yet available and the knowledge of nuclear genes with known function is still very limited, representing only 1.47% of the sequences present in the database [9].

A list of sequences is available on the website of NCBI: 16692 nucleotide sequences, 1868 EST and 4797 GSS, almost all (11203) belonging to *Bubalus bubalis* species, followed by *Bubalus bubalis* bubalis (107) and other taxa.

The lack of the gene annotation is reflected also in the very limited information for the genetic variability, which represents the first step of the knowledge for the genetic improvement of the species.

The total number of available reference SNP reported on NCBI data base is only 502 for this species (http://www.ncbi.nlm.nih.gov/snp/?term=bubalus+bubalis) and the validation status of these polymorphisms is in most cases missing. Considering the close distance between *Bos taurus* and *Bubalus bubalis*, [10] employed the Illumina Bovine SNP50 BeadChip in buffalo. Although most of SNP were fully scored (41870 *vs* 54001), only 1159 SNP were polymorphic in the species.

The conservation of the SNP sites but not of the polymorphisms between cattle and buffalo indicates that as long as the buffalo genome and its annotation is not complete, the use of already existing tools for the genetic improvement of the species is not useful at all. Therefore, the application of genome wide association studies (GWAS) is still very far to be a reality as for instance happens in cattle, and a classical research approach based on marker assisted selection (MAS) or gene assisted selection (GAS) seems to be still far away for the planning and the application of breeding selection schemes.

2. THE ITALIAN SITUATION

The buffalo reared in Italy belongs to the Mediterranean Italian breed, and it is different from other river types reared in Europe. Although these breeds belong to the same lineage, they are characterized by a different genetic level [11]. Demography data show that the Buffalo population in Italy is a small reality compared to the huge populations of the East Asian countries. Despite that, the Italian buffalo population has increased 19 fold in the past fifty years (http://faostat.fao.org), becoming the livestock that has registered the highest increase (together with Brazil) in the world among the years 1961-2011 (Table **1**).

CHAPTER 4

Animal – Environment Interaction: Buffalo Behavior and Welfare

Fabio Napolitano[1,*], **Corrado Pacelli**[1], **Ada Braghieri**[1], **Fernando Grasso**[2] and **Giuseppe De Rosa**[2]

[1] *Scuola di Scienze Agrarie, Forestali, Alimentari ed Ambientali, Università della Basilicata, Via dell'Ateneo Lucano 10, 85100 Potenza, Italy*

[2] *Dipartimento di Agraria, Università di Napoli Federico II, Via Università 133, 80055 Portici (NA), Italy*

Abstract: This chapter will focus on the effects of interaction of river buffaloes with the environment both in confinement and in extensive conditions, on their behavior and welfare. Firstly, the time and place of domestication are described. Sections on time budget, foraging and feeding habit, maternal and social behavior, and some aspects of style of interaction with the environment and temperament are then elucidated. Subsequently, focus is shifted on the quality of relationship between stock-people and animals, which is an important aspect affecting animal welfare in modern farms. Finally, the welfare consequences of intensive farming on buffalo welfare are evaluated using a number of animal-based indicators.

Keywords: Animal welfare, Behavior, Dairy buffalo, Housing system.

1. INTRODUCTION

The wild species belonging to the genus Bubalus were widely distributed in Asia and Europe during the Pleistocene. The subsequent climate changes restricted the area of distribution of wild species to the Asian continent, from India to the far South-East. The corresponding domestic animals are generally referred to as

---

[*] **Corresponding author Fabio Napolitano:** Scuola di Scienze Agrarie, Forestali, Alimentari ed Ambientali, Università della Basilicata, *Via* dell'Ateneo Lucano 10, 85100 Potenza, Italy; Tel: +39 0971 205078; Fax: +39 0971 205378; E-mail: fabio.napolitano@unibas.it

Giorgio A. Presicce (Ed.)

water buffaloes and grouped in two main classes on the basis of their genetic make-up and phenotype. The river buffaloes present 50 chromosomes, and are currently widespread in Italy, few eastern European countries, middle east and India; the swamp buffaloes present 48 chromosomes and are mainly found in South-East Asia (China, Bangladesh, Vietnam, *etc.*) [1]. The time and place of domestication of buffaloes have been debated for a long time but recently, Kumar *et al.* (2007) proved that river and swamp buffaloes were independently domesticated [2]. In particular, all of the buffaloes belonging to the river type (including both Indian and Mediterranean animals) are likely to be derived from the same stocks [3]. This sub-group was domesticated around 6300 years BP, whereas archaeological studies identified the South-East of India as the place of domestication [3]. Most likely in the period around 2600 BP, water buffaloes were taken by Arabs from Mesopotamia to the Near East. During the Middle Ages, pilgrims and crusaders possibly brought the domestic animals back to Europe. There, buffaloes were reared in extensive conditions for centuries, whereas nowadays they are predominantly kept in intensive conditions. This chapter will deal with the expression of river buffalo behavior, in intensive and in extensive conditions, and will subsequently focus on how different housing systems and management practices can affect the welfare of these animals.

2. BEHAVIOR

2.1. Time Budget in Extensive Conditions

In extensive conditions in ruminants, time is allocated and spent on different behaviours, according to stocking density, nutritional requirements as well as availability and distribution of food together with the perceived threat of possible predators. A significant amount of data is available on time budget of cattle, sheep and goats in extensive temperate and tropical zones [4, 5], whereas little is known about the allocation of the time performed by the buffaloes. The wild African buffalo (*Syncerus caffer*) at the Kruger National Park (South Africa) has been studied with regard to the analysis of its diurnal activity, and it has been shown that these buffaloes spend approximately 40% of the time grazing, followed by 30% resting and 30% ruminating [6]. Accordingly, in Uganda buffaloes of the same species spend 9 h a day grazing and 6.4 h a day ruminating [8]. Conversely, the feeding time of Cape buffalo (*Syncerus caffer caffer*) covers 52% of the daytime with peaks observed in the morning and in the evening, whereas resting has been more often observed at midday [7]. In Italy buffalo farming has been run extensively for a long time. Buffaloes are still characterized by some physiological and morphological features acquired through natural selection, which are then capitalized to reinforce their ability to thrive well in open environments (*e.g.* reproductive seasonality, melanin-pigmented skin). Recently, an experiment on maintenance behavior of buffalo heifers [9] was conducted in a fenced Mediterranean maquis located in Southwest Italy. At the beginning of the experiment, animals were about 8 to 9 months of age. They were free to graze and continuous focal animal sampling was used to collect behavioural data [10] from April to October. The activities displayed by the buffaloes during 6-h observation periods are depicted in Fig. (**1**). The behavioural pattern of other ruminants reared in extensive environmental conditions was similar [4].

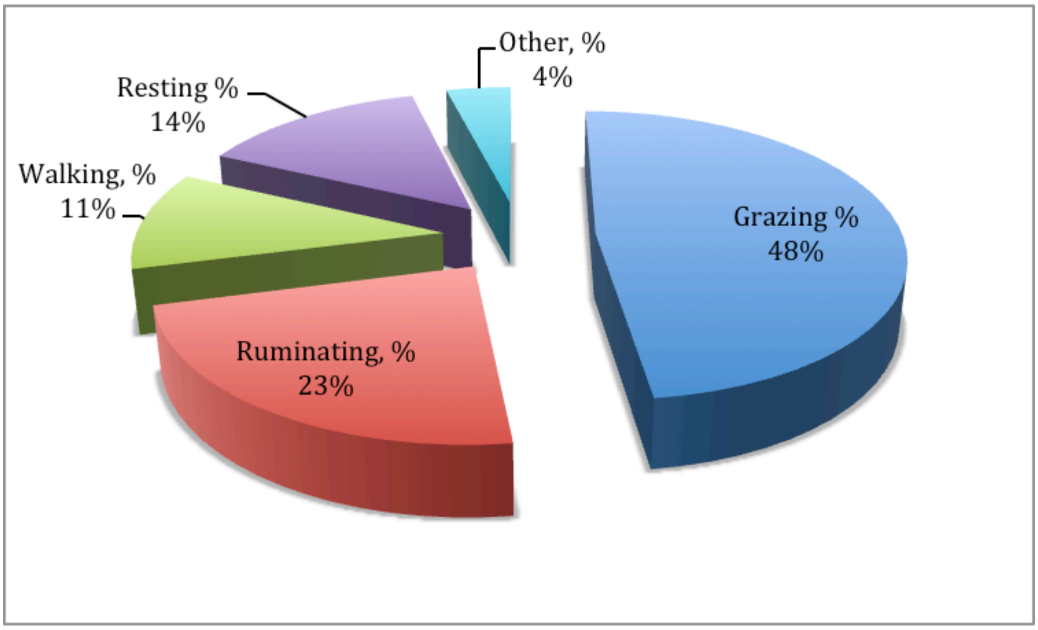


Fig. (1). Behavioral pattern (%) of buffalo heifers observed over 6-h periods in extensive conditions [9].

No significant effect of season on the distance covered by the animals was found by authors, although a longer distance was run in spring time (Table **1**). Ryan and

CHAPTER 5

Thermal Balance in the Buffalo Species

Ramesh C. Upadhyay[1,*] and **Narongsak Chaiyabutr**[2]

[1] *Dairy Cattle Physiology Division, National Dairy Research Institute, Karnal-13200, Haryana, India*

[2] *Department of Physiology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand*

Abstract: Buffalo maintain thermal balance by physiological and behavioural processes. During heat stress, on exposure to direct solar radiation or during work, buffaloes exhibit signs of distress. Under extreme hot dry or hot-humid environmental conditions ability of buffaloes to regulate temperature is compromised, and body heat balance that is dissipated at later stage or during cool periods by heat exchange processes, is increased. The responses of body functions under either acute or short term heat are discussed in relation to the initiation of panting by thermal stimulation of peripheral receptors and to the control of respiratory activity by deep body temperature. During heat exposure, an increase in water turnover reflects adaptation to maintain fluids for evaporative cooling. Acute exposure or short term exposure to heat evokes several responses of plasma volume and its composition, including changes in potassium metabolism. Acute heat exposure gives rise to a change in renal hemodynamics and electrolytes excretion. Changes in renal electrolyte excretion during heat stress are discussed in relation to the alteration of hormonal level and to the acid-base status of the blood. The effect of heat exposure on various other reactions is also summarized in thyroid activity, the levels of hormones in the pituitary and adrenal glands.

Keywords: Buffaloes, Heat exchange, Responses of body functions, Thermoregulation.

---

[*] **Corresponding author Ramesh C. Upadhyay:** Dairy cattle physiology division, National dairy research institute, Karnal-13200, Haryana, India; Tel: +91 184 225 9023; Fax: +91 184 225 0042; E-mail: ramesh5304@rediffmail.com

Giorgio A. Presicce (Ed.)

I. FACTORS AFFECTING THERMAL BALANCE OF BUFFALOES

1. INTRODUCTION

Buffaloes, though well suited to hot and humid climates and muddy terrain because of their morphological and anatomical characteristics, yet exhibit signs of great distress on exposure to direct solar radiation or during work in the sun. Buffaloes maintain their homeothermy *via* various physiological and behavioural mechanisms under normal comfortable environmental conditions. Under extreme hot dry or hot-humid environmental conditions, thermoregulatory ability of buffaloes is compromised and they are unable to maintain core body - HYPERLINK http://en.wikipedia.org/wiki/Core_temperature\o Core temperature - temperature within physiological limits. This process is one aspect of - HYPERLINK http://en.wikipedia.org/wiki/Homeostasis\o Homeostasis - homeostasis in buffaloes and a dynamic state of stability between an animal's internal environment and its external environment.

2. TYPES OF THERMOREGULATION

Buffaloes have been observed to use two types of thermoregulation:

i. Physiological Regulation**:** Physiological regulation of body temperature in buffaloes induces sweating to cool body down and exposure to cold induce shivering in young and adult buffaloes. Young buffalo calves and neonates shiver to keep warm and generate heat.

ii. Behavioural Regulation**:** Buffalo changes its behaviour to regulate body temperature particularly during extreme hot and cold climatic conditions. Under hot dry conditions, buffaloes avoid direct solar exposure and seek shade. Under hot and hot- humid weather, when body starts to get hot because of the intense solar radiations, buffaloes may want to seek a shade to reduce direct exposure and cool down or prefer wallowing. Buffaloes have unique ability to regulate body temperature by wallowing.

A. Core and Skin Surface Temperature

The core body temperature of buffaloes is 36.5° C and remains within ± 1° C of such value during normal comfortable ambient conditions, but it may increase by several degrees under stressful hot climatic condition.

Buffalo exhibit a diurnal variation in body temperature. In general body temperature is minimum in the early morning and maximum in the late afternoon. Diurnal variations occur in core temperature of buffaloes, and the evening temperature is about 0.5-1.0 ^{0} C higher than early morning body temperature. The rectal temperature of buffaloes increase by 2-3^{0}C under direct solar exposure, and magnitude of rise may be higher in working buffaloes during hot conditions. This variation largely reflects activity and feeding during the day and rest during the night.

B. Skin Characters

In general buffalo skin colour is dark black; however, skin colour may vary with the condition of management. It has been observed that housing buffaloes may cause a loss of their skin pigmentation which may be regained following further exposure to solar radiation. Pigment is brown or black in colour and is restricted to the cytoplasm of the epidermal cells. The density of the pigment is proportional to the exposure of the skin to sunlight and due to this reason dorsal and ventral areas, which are more exposed to sunlight, exhibit greater pigmentation than ventral and less exposed parts [1]. The most striking feature of buffalo skin is the thickness of the epidermis, which may be 1.5-2% of the total skin thickness. The horny layer (*Stratum corneum*) is very conspicuous and may be twice thick as in cattle. The typical black color of the buffalo skin surface is given by the melanin particles contained in the basal cells of the epidermis [2]. The melanin particles are more numerous in skin from the dorsal region of the buffalo and less numerous on the belly and the inner surface of the thigh [1]. The ultraviolet rays are trapped by the melanin particles, interrupting thus penetration of the same rays through the dermis and into deeper tissues. The tropics and subtropics are characterized by abundant ultraviolet rays, and a detrimental effect can be foreseen if an excessive exposure to those rays is not avoided, with the result of even skin tumors. The thick epidermis and *stratum corneum* may be beneficial to the buffalo as they protects the sparsely covered or nearly bare skin surface of buffaloes from harmful mechanical and chemical agents. The skin of buffaloes

CHAPTER 6

Feed Resources, Rumen Fermentation, Manipulation and Production in Swamp Buffalo: A Review

Metha Wanapat[*], **V. Chanthakhoun, R. Pilajun** and **P. Khejornsart**

TROFREC, Faculty of Agriculture Khon Kaen University Khon Kaen 40002, Thailand

Abstract: Swamp buffaloes (*Bubalus bubalis*) are multipurposes and resourceful animals in agriculture of incredible importance among farmers. Within a smallholding farming system, typical of many Asian countries, seasonal feed resources used on swamp buffaloes, are of paramount importance for an efficient production. In fact, during the dry season, when conventional feed resources are usually scarce, their contribution is incredibly relevant. In addition, the utilization of such feed resources may represent an efficient way to reduce methane production through better use of secondary compounds in tropical plants and herbs (tannins, saponins, *etc.*), and consequently improve the overall rumen ecology and finally buffalo productivity. Currently, the development of food-feed systems (FFS) have been successfully implemented and therefore should be fully integrated into the ordinary use by smallholder farmers. However, in order to improve and make more efficient feeding methodologies, both treatments and/or supplementations should be considered, such as the development of simple and practical feeding and the use of concentrate mixtures based on on-farm resources (home-made concentrates, HMC) in order to reduce production costs and enhance profitability and sustainability of the buffalo production. The manipulation of rumen fermentation by treating roughage and/or by supplementing the available feed resources with plants characterized by high quality feed block, tannin/ saponins, especially cassava hay and other local feed resources, could improve rumen efficiency by maintaining a constant higher pH, optimum NH_3-N, and increasing microbial protein synthesis and essential VFAs, and therefore enhancing ruminant productivity in the tropics. Moreover, buffaloes have been shown to be more efficient in feeds utilization, when compared to cattle. Lately, the application of molecular

---

[*] **Corresponding author Metha Wanapat:** TROFREC, Faculty of Agriculture Khon Kaen University Khon Kaen 40002, Thailand; Tel: +66 819324180; Fax: +66 202362; E-mail: metha@kku.ac.th

Giorgio A. Presicce (Ed.)

technology to rumen studies, such as the use of PCR-DGGE and real-time PCR, has been instrumental in offering a wide range of information and data on rumen microbial diversity and the likelihood of a possible functional role in reducing rumen methane as well as enhancing productivity in swamp buffaloes.

Keywords: Buffaloes, Feed resources, Feeding, Integrated livestock system, Methane production, Rumen ecology manipulation.

1. INTRODUCTION

Swamp buffaloes are closer to wild ruminants than their bovine counterparts and have played a very important role in providing draught power, manure as fertilizer and meat for people. An important aspect for ruminants within the rumen ecology, that is well established and understood among researchers, is the function of fermentation within the rumen itself that allows the preparation of end-products, and particularly volatile fatty acids (VFAs) as well as microbial proteins, to be used as a major energy and protein drive for the ruminant host. However, crop residues are definitely the main feed resources available to ruminants, usually by-products derived from the agro-industrial productive systems utilizing native grasses from fertile lands, which, in general, support only low levels of production. This is particularly true in the tropics where, both buffaloes and cattle are raised as an integral part of crop production, and where rice is the most important available commodity, from which the related by-products are employed as feeds [1]. Some local feed resources though, like cassava root/hay/silage, corn stover, leucaena leaves, sweet potatoes vines, sugarcane tops, *etc*., can be considered potential additional feed resources for ruminants which are capable to enhance production efficiency. Usually ruminants are fed roughages, like crop residues, such as rice straw, wheat straw, and maize stalk, and from this use, a significant amount of methane is produced and emitted [2]. In the tropical environment, a large amount of plants contain secondary compounds such as saponins, condensed tannins, essential oil, and plant extracts, which are known to effectively inhibit ruminal methanogenesis [3 - 5]. On the other hands, these same forages have been shown to increase protein availability in the small intestine of ruminants [6]. In addition, cassava and legumes usually contain secondary compounds which could have an effect on rumen fermentation and productivity, whereas rumen bacteria can further detoxify these compounds. It has been reported by Hegarty [7] that a significant reduction of methane production per gram of live weight gain is achieved when animals are shifted from a low to a high digestible pasture.

Due to the lowering of world prices for plant commodities and the projected future increase of animal products caused by higher demands [8], it is possible that a realistic objective may be given by the contribution of food-feed system (FFS) to ruminant production, particularly when in need to review their overall high nutritive values, the positive effects on rumen, as well as the expansion and intensification of these systems. It has to be emphasized that FFS has received over time more and more attention and interest by farmers working within a smallholding system. Historically, the majority of knowledge on rumen microbial composition has been acquired by employing traditional methods such as roll tube technique [9] or most probable-number (MPN) estimates [10]. Some microorganisms though, cannot be cultivated under current technology, although their artificial culture represents only a small fraction of natural microbial communities, and as a consequence, the microbial diversity is grossly under-estimated [11].

Nowadays, microbial diversity found in complex ecosystems like the rumen, has been studied using new gene-based technologies [12]. Both DGGE and real time PCR are culture independent techniques that target that part of DNA encoding the synthesis of small subunit ribosomal RNA (SSU rDNA), which allow a direct analysis and quantification of microorganisms of interest [13]. Such data can offer very useful information and provide the possible functional roles of microorganism themselves in the rumen of swamp buffaloes.

2. SEASONAL FEEDING SYSTEMS FOR RUMINANTS

A seasonal approach to ruminant feeding in a tropical environment can be split into a dry and a rainy seasons, although a reduced productivity can be reported due to an improper feeding supply and regimen with regard to both quantity and quality [14]. Despite its low nutritive value, rice straw has been typically used during the dry season as an ordinary feeding approach, generally practiced by

CHAPTER 7

Protein Digestion and Metabolism in Buffalo

Vo Thi Kim Thanh[1,*] and **Egil Robert Orskov**[2]

[1] *Department of Animal Physiology, Hue University of Agriculture and Forestry, Vietnam*

[2] *International Feed Resource Unit, Macaulay Land Use Research Institute*

Abstract: As other ruminants, buffaloes utilize micro-organisms in the rumen to digest the feed. In buffaloes a higher rumen degradability of nitrogen and carbohydrates in concentrates promotes the growth and the synthesis of rumen bacteria, even when fed diets with low protein content. It appears that buffaloes use more efficiently nitrogen coming out from rumen fermentation and metabolism and by recycling it. This efficient accommodation to more limiting feeding condition is enhanced by a higher availability of purine derivatives (PD) of metabolic origin. Many measurements done on buffaloes of various breeds have shown a lower PD nitrogen excretion in urine. In buffaloes, urinary PD excretion is not linked to i) the availability of microbial cells in the rumen or ii) small intestine uptake of purines. Such PD excretion seems to be related more to tissue metabolism differences of which the mechanisms are not yet fully understood. Some explanation is emerging with new studies on swamp buffaloes, summarized in the following two: i) in the first study, weaning of swamp buffalo and cattle calves was accomplished by colostrum administration, and rearing followed by milk bottle feeding. To assess differences in the endogenous secretion of purines, urine samples from the two species were collected. Solid food was not made available in the course of the first month, but access to it was granted in the course of the intervening 2 successive months in order to stimulate rumen development. Then a mixed ration of purines and milk was given to the animals, together with an infusion of intravenous allantoin, so that the effect of the introduced purines in the plasma could be tested. From the results obtained in the course of the suckling period, no differences between the two species in purine excretion was reported. Following rumen development though, purine excretion from buffaloes was less than half when compared to cattle, and likewise, following allantoin infusion, purine recovery in buffaloes was half the

---

[*] **Corresponding author Vo Thi Kim Thanh:** Department of Animal Physiology, Hue University of Agriculture and Forestry, Vietnam; Tel: +84 914 192 906; Fax: +84 54 3 524 923; E-mail: votkthanh@gmail.com

Giorgio A. Presicce (Ed.)

amount when compared to cattle ; ii) in the second study in the course of fasting and bottle milk feeding, a determination of urinary PD, basal PD excretion and glomerular filtrate (GFR) rate was made. Following access of the animals to solid feed, an assessment of urinary PD, basal PF excretion and glomerular filtrate rate was also performed. No significant differences were observed between the two species in the course of the milk feeding period in terms of urinary PD excretion, although the same differences were highly significant between the two species at 3 months of age and following 2 months of access to solid feed. In buffaloes, both during the milk feeding and following solid food access, GFR was found lower in buffaloes when compared to cattle. To date it can be stated that some studies report a higher rumen fluid NH_3 concentrate in swamp buffaloes in comparison to yellow cattle. Other studies have shown that only following rumen development, a difference in PD excretion can be seen, and the difference between buffaloes and cattle is due to differences in GFR, so that more urea and PD are recycled, highlighting the fact that buffaloes can tolerate less N in the feed to satisfy microbial needs.

Keywords: Buffaloes, Feeding, GFR, Microbial protein, Purine derivative, Rumen, Rumen NH_3.

1. INTRODUCTION

Feed for ruminants, forages and fibrous roughages, consist mainly of β-linked polysaccharides such as cellulose, which cannot be broken down by mammalian digestive enzymes. Ruminants have therefore evolved a special system of digestion that involves microbial fermentation of feed prior to its exposure to their own digestive enzymes [1]. The rumen microbes can use simple compounds such as ammonia and urea, and build up their cells from them. In fact, they can use any compound which will be degraded into ammonia – even urine. Microbial proteins then provide most of the animal protein need. So in effect, it is not necessary to feed the ruminant animal any protein at all since the microbes in the rumen can provide for them. And almost all the protein in feeds can be degraded by the bacteria. A bigger problem arose when it was shown that nearly all protein in the dung consisted of indigestible microbial cells, whereas only very little protein from the diet was truly indigestible protein. Therefore, if microbial growth occurred in the hind gut of animals, the microbes were not digested at all but passed out in the dung. This of course meant that the measured digestibility had very little meaning as far as actual digestibility of feed protein was concerned [2].

Therefore, the most important source of protein supply for ruminants is constituted by microbial population within the rumen. Nucleic acids are abundant in such microbes, and they possess roughly 18% of the total amount of nitrogen, whereas purines are characterized by a total nitrogen content of about 11%. Rumen microbial population metabolize purines, followed by urine excretion of their end products such as hypoxanthine, xanthine, allantoin and uric acid. That is why the research work has been carried out actively for the past 20-30 years into the urinary excretion of these purine metabolites, with an objective to use the excretion of these metabolites as a parameter to quantitatively estimate the supply of rumen microbial protein to the ruminant.

2. BUFFALO FEEDING AND PROTEIN DIGESTION

Feeding

Buffaloes are ruminants, and ingested feed, which is essentially made of vegetables of various kind, is metabolized in the rumen by micro-organisms, which efficiently convert cellulose and other fibres into high quality milk and meat. Furthermore, it seems that buffaloes are more efficient in using poor quality feed such as rice straw, crop residue or other by-products. Management practices in Asia, such as in Vietnam, Thailand, Lao, Cambodia, China, are run through the adoption of extensive systems in which buffaloes freely graze on natural grasses and marginal lands (roadside, canal banks, rice field following harvesting and dikes), as well as forests, among other conditions.

Rumen Digestion

Although rumen transit of the liquid phase is similar in cattle and buffaloes, the transit of the solid fraction is significantly slower in buffaloes. This difference though is compensated during the post ruminal tract so that, if referred to the overall digestive tract, the feed transit is faster. One must take into account that rumen volume in buffaloes is higher (about 10%) than cattle at the same live weight [3]. The feed transit accounts for the differences observed of rumen degradability of feed fractions. The protein rumen degradability of forages and concentrates is significantly higher in buffaloes than in cattle [3] and sheep [4]. The higher nitrogen degradability affects the ammonia N content of the rumen,

CHAPTER 8

Influence of Seasonality on Buffalo Production

Luigi Zicarelli[*]

Dipartimento di Medicina Veterinaria e Produzioni Animali - Università "Federico II" – Napoli, Via Delpino, 1 - 80137 Napoli, Italy

Abstract: This review aims at elucidating some of the factors that affect seasonality in the buffalo species, together with the possibility to enhance reproductive performance in buffaloes through the adoption of newly developed technologies. It is known that reproduction in buffaloes is influenced by the season, and an improvement in reproductive performances is reported in the period of the year when the length of the day decreases. If conception is not established in buffaloes in the course of few ovarian cycle, ovarian function is interrupted and a period of ovarian quiescence (anestrus) begins. The transitional period in the buffalo is an important one for reproductive functions, and throughout this period a proper management of the animals has to be taken into consideration, especially in order to properly maintain the hygienic status of the uterus. Buffaloes reared in tropical countries north of the equator may be characterized by a reduced fertility in the summer when hit mainly by restricted feeding and heat stress. In some countries like Pakistan, the breeding season starts in a period of the year characterized by decreasing daylight (autumn), together with an increase in body condition score. Differently, despite a constant feed availability in the course of the year and a moderate daily temperature ranging from 13 to 23 °C, anestrus can be witnessed also in Italy. These two countries are similarly characterized by an increase in daylight from April to June and reaching more than 12 hours of light hours at summer peak. In order to improve fertility in Italian buffalo herds, an increasing number of farms adopt the Out of Breeding Mating Strategy (OBMS) together with the availability of water pools. This is a clear evidence that reproductive performance as a whole can be improved, together with a reduction in the incidence of embryonic mortality and ovarian inactivity, when environmental conditions are also improved.

---

[*] **Corresponding author Luigi Zicarelli:** Dipartimento di Medicina Veterinaria e Produzioni Animali - Università "Federico II" – Napoli, *via* Delpino, 1 - 80137 Napoli, Italy; Tel: +39 081 2536800; Fax: +39 081 292981; E-mail: zicarell@unina.it

Giorgio A. Presicce (Ed.)

Keywords: Buffalo, Reproduction, Reproductive technologies.

1. INTRODUCTION

The official FAO data refers that buffaloes are present in 43 countries and are hence bred in 20% (43 *vs.* 212) of the countries in which cattle is bred. On the contrary, countries that currently breed buffaloes are 129 [1] and hence it is likely that this species is bred in 61% of the countries around the world (129/212). Cattle world population (Faostat.fao.org/faostat) moved steadily from 942 millions in 1961 to 1.494 billions (+ 59%) in 2013, and within the same time interval, buffaloes increased from 89 millions to 199 billions (+ 125%). Between 1990 and 2013, the percentual increment in those countries where the two species are bred, has always been higher for the buffalo species (Table **1**). It has to be emphasized though, that most of the countries where the two species are bred, are in Asia, where the human population has considerably increased, and such aspect justifies the increment.

Table 1. Buffalo and cattle population trend (%) between 1961 and 2013.

	Cattle		**Buffalo**	
World	2013	1961	2013	1961
buffalo and cattle population trend (%) between 1961 and 2013 (x 10^6)	1494 (59)	942	199 (125)	89
buffalo and cattle population trend (%) between 1990 and 2013 (x 10^6)	1494 (15)	1297	199 (35)	148
countries where two species are bred	2013	1961	2013	1961
buffalo and cattle population trend (%) between 1961 and 2013 (x 10^6)	705 (87)	378	199 (127) *	88
buffalo and cattle population trend (%) between 1990 and 2013 (x 10^6)	705 (29)	547	199 (35*)	148

*This increase is mainly due to the increase of River buffalo (50 n; milk production), while the Swamp (48 n; draught animal power), decreased by 27% [2].

The buffalo represents a fundamental and irreplaceable resource for tropical countries. The increase in the number of buffalo heads is mainly due to the increase of River buffaloes (50 n), utilized for milk and meat production, while the Swamp buffaloes (48 n), mainly used as draught animal power, has decreased by 26.69% [2], especially in South-Eastern Asia. The Swamp buffalo in many countries is crossbred with the river type, due to an increase in milk demand. In fact, it is true that the dairy cow is not always able to totally exploit its genetic merit for many months in the tropical areas, due to the high temperatures and high humidity rate. On the contrary, under the same conditions, the River buffalo can still support an optimal production, although, it retains a sensitivity to such environmental conditions. In fact, if nutritive requirements are satisfied, buffaloes are characterized by similar milk productions in both tropical and temperate areas. In the last years, world's buffalo milk percentage has increased from 5 to 14.8%, but such values can bounce from 8.3 to 21.6%, when we consider that buffalo milk is 58% higher in energy than its cattle counterpart.

In Southern Asia, where 78% of the world's buffalo population is bred, in cattle, and compared to the year 1961, a higher milk increase has been observed (+ 657% *vs.* + 581%). This phenomenon can be explained by the use of imported superior genetic gametes and embryos from industrialized countries in order to obtain an improvement in animal production. However, the increase in milk production alone is not sufficient to justify the decision to increase the number of an animal species, or to consider it more economically viable and efficient. Other factors have to be considered, such as: lower susceptibility to diseases, higher adaptability to the tropical environment, higher herd life, and hence, a lower culling rate. These conditions will favour an increase in the number of heads within the herd also for beef production, matching perfectly production and forage availability. In fact, in South Asia, lactation in buffaloes coincides with an abundance of forage availability, while the dry period will match the quiescence of the vegetative cycle. These are fundamental aspects that reduce the advantage in cattle linked to their higher production performance. In particular, and with regard to ordinary culling rate between the two species, if we consider a breeding farm of 100 females in production, it is necessary to breed around 90 (26% culling rate) and 42 (12% culling rate) heads (adult animals), in cattle and buffalo, respectively. Consequently, the same number of cattle heads will need a much larger land when compared to buffaloes, and in those countries where farms are characterized by low size, this difference is economically important.

In India in 1991, buffalo milk consumption was 55% of the total milk produced in

CHAPTER 9

Buffalo Dairy Production: A Review

Marco A. Zava[1,*] and **Marina Sansinena**[2,3]

[1] *Technical Department of Argentine Buffalo Breeders Association; International Consultant, Arcos 1325, 1426, Buenos Aires, Argentina*

[2] *Animal Biotechnology Laboratory, College of Agriculture, Catholic University of Argentina, Cap. Gral. Ramón Freire 183, 1426 Buenos Aires, Argentina*

[3] *National Council for Research and Technology (CONICET), Buenos Aires, Argentina*

Abstract: Worldwide production of buffalo milk is steadily increasing. In this chapter, the worldwide distribution of buffalo milk production is described, with emphasis on production styles and characteristics by region. Representative buffalo breeds are described, along with production levels and general herd management. Relevant factors such as heat stress, animal nutrition, conversion efficiency, health management, calf rearing systems and sanitary milk production conditions are outlined. Factors influencing buffalo milk performance and production are also outlined in this chapter such as sexual maturity, calving interval, days in lactation, residual milk and performance according to age groups.

Keywords: Breeds, Buffalo, Dairy, Production levels, Worldwide distribution.

1. INTRODUCTION: WORLDWIDE DISTRIBUTION, REGIONAL PRODUCTION AND ECONOMY

Worldwide Distribution

Worldwide production of buffalo cow milk is steadily increasing, and is concentrated in three countries which represent 94% of the total world production: India, Pakistan and Nepal [1]. In addition, significant amounts of

---

[*] **Corresponding author Marco A. Zava:** Technical Department of Argentine Buffalo Breeders Association; International Consultant, Arcos 1325, 1426, Buenos Aires, Argentina; Tel: +54 011 4706 2513; Fax: +54 11 4373 9982; E-mail: marcozava@gmail.com

Giorgio A. Presicce (Ed.)

buffalo milk are being produced by China and Egypt (in the latter, bubaline milk production is higher than cattle) [2].

Of the total world buffalo population, 69.73% is found in India and Pakistan and it is mainly dedicated to dairy production [3]. According to FAO 2008 [3], India and Pakistan produce 66.62% and 25.15% of the buffalo cow milk worldwide, respectively. In 2008, India produced 56.960.000 metric tons of buffalo cow milk and Pakistan produced 21.500.000 [3]. India is the largest producer of buffalo heads in the world and also the largest milk producer (cattle + bubaline, approximately 134 million tons). Only 15% of buffalo cow milk is processed by dairy plants, while the rest is consumed as raw milk for human consumption [5].

Buffaloes play a key role in the agricultural economy of the Indo-Pakistani continent. This species is known for being an efficient converter of low-quality forages and by-products into a single valuable commodity: milk. An example to understand the magnitude of the economic importance of bubaline milk production in India: although the 106,63 million buffalo heads [3] represent only 34.35% of total livestock in the country, their milk output accounts for 65% of total dairy and dairy by-products [3, 4, 6].

In the year 2004, bubaline milk accounted for the 12% of the world dairy production (75.833.191 tons of bubaline milk *versus* 608.943.729 total). Between 1996 and 2004, bubaline milk production increased 26%, *versus* only a 10% increase in vaccine milk. According to FAO, in the last three decades worldwide bubaline production increased 200% [4], with an annual growth of 7%. This growth is mainly attributed to the 110% increased milk production of Asia in the last two decades (India 101%, Vietnam 93% and Pakistán 88%) [3], and the continent is now producing 96% of the world bubaline milk. In 2008, Brazil produced 114 million liters and Italy 200 million (600 million euro) [4]. Production of several key countries is shown in Table **1**.

Table 1. Top producers of buffalo cow milk in 2008.

Country	Production (Tons)
India	56,960,000
Pakistan	21.500.000

(Table 1) contd.....

Country	Production (Tons)
China	2,900,000
Egypt	2.300.000
Nepal	930,000
Iran	241.500
Myanmar	205,000
Italy	200.000
Brazil	114,000
Turkey	35.100
Vietnam	31,000
WORLD TOTAL	**84,488,902**

Source: Otavio Bernardes, 2010 [4]

Regional Production and Economy

Europe

In Italy, buffalo industry is an intensive production system that utilizes the latest technology, with more than 60 years of genetic selection and quality control. Average milk production per lactation has increased over 400 kg since the national genetic improvement program started in 1969. In superior herds, lactations of 5000 liters for 250-day lactations with two daily milking sessions can be achieved and for the Mediterranean breed, lactations of 240-270 days with values of 1500, 2700 and 6000 liters have been reported. In 2009, Naples reported 3000 daily kilograms of mozzarella cheese exported to the U.S.A. and Great Britain. Bulgaria has reported productions over 3500 liters, mostly switching from Mediterranean to Murrah breeds.

Seliano Farm, owned by the Bellelli family, in Seliano, Salerno Province, Campania Region, Italy, produces corn, ryegrass, oats and olive trees over 90 hectares of land, 7 of which are occupied by animal facilities. Of a total of 768 buffaloes, 338 are adult females. In the year 2004, they reported a total of 544.200 kg of milk from 220 animals in milking, with an average 2500 kg/lactation, 4.8% crude protein and 8.5% butirose fat. This farm operates non-seasonally (out of season mating). Fifty-six percent of the milk was produced in the summer season

CHAPTER 10

Buffalo Milk Characteristics and By-Products

Marco A. Zava[1,*] and **Marina Sansinena**[2,3]

[1] *Technical Department of Argentine Buffalo Breeders Association; International Consultant, Arcos 1325, 1426, Buenos Aires, Argentina*

[2] *Animal Biotechnology Laboratory, College of Agriculture, Catholic University of Argentina, Cap. Gral. Ramón Freire 183, 1426 Buenos Aires, Argentina*

[3] *National Council for Research and Technology (CONICET)*

Abstract: This chapter covers a review of chemical composition and nutrient profile of buffalo milk; several key physical properties (freezing point, surface tension, electric conductivity and thermal stability) are described. Regional manufacturing, utilization and marketing of buffalo milk by-products such as cheese, yogurt, ghee and others are also described and step-by-step flow charts are presented.

Keywords: Breeds, Buffalo, Buffalo milk by-products, Chemical composition, Dairy.

1. INTRODUCTION

Products derived from fluid bubaline milk, such as butter and ricotta, frescal and mozzarella cheese, have outstanding attributes in terms of product quality and health benefits. Other products, such as fluid milk or ghee, have been adapted by some countries in order to satisfy local market preferences and specific human nutrition demands. In all cases, fluid buffalo milk and its by-products provide excellent nutrient profiles and unique attributes for human nutritional needs; this translates into a wide range of products with high palatability, outstanding cheese yield, and unique health benefits.

---

[*] **Corresponding author Marco A. Zava:** Technical Department of Argentine Buffalo Breeders Association; International Consultant, Arcos 1325, 1426, Buenos Aires, Argentina; Tel: +54 011 4706 2513; Fax: +54 11 4373 9982; E-mail: marcozava@gmail.com

Giorgio A. Presicce (Ed.)

2. BUFFALO MILK COMPOSITION AND NUTRIENT PROFILE

Buffalo cow milk quality is considered to be excellent due to its unique nutrient profile. It is an excellent source of protein, vitamins, minerals and other constituents and it provides calories in countries where the overall supply of essential nutrients is deficient. Bubaline milk is higher in fat, protein, lactose and total solids than milk from cattle (Fig. **1**). Its fat content is 2.5 to 3 times higher and it provides 40% more calories than its cattle counterpart [1].

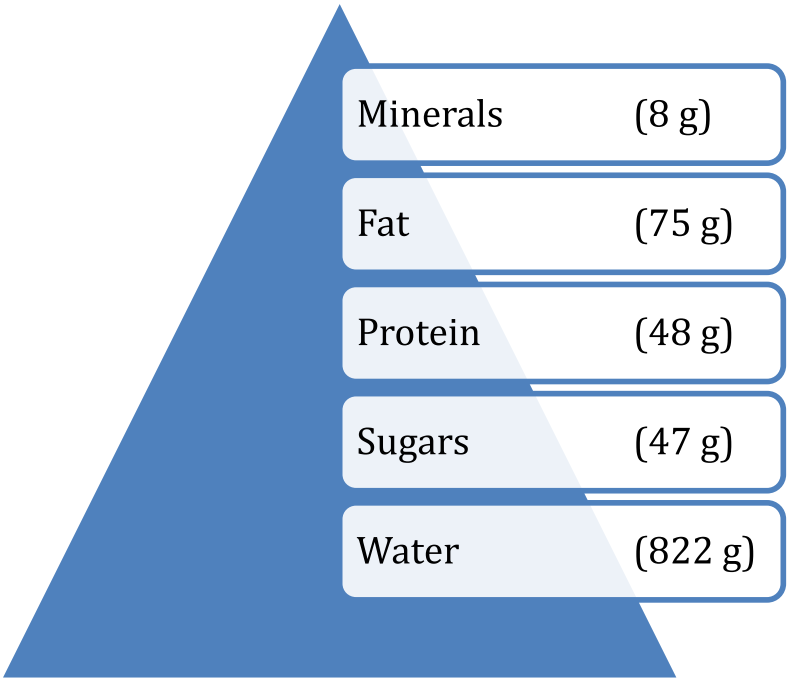


Fig. (1). Average composition of buffalo milk [3].

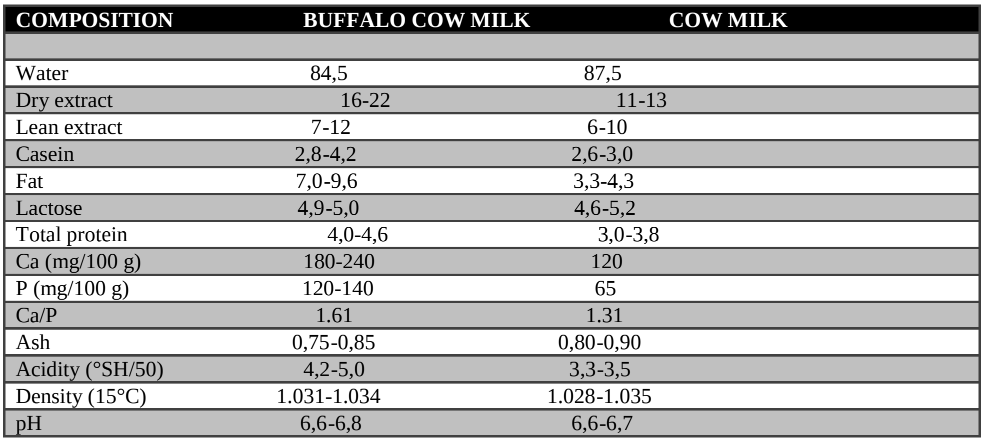
Studies conducted in Brazil with Murrah breed showed that buffalo milk composition includes conjugated linoleic acids (CLA), considered as anti-carcinogen compounds and shown to have beneficial effects on health problems such as obesity, arteriosclerosis and diabetes. This study also demonstrated that supplemental feeding of raw soybean oil further increases CLA content in buffalo milk [2].

The main differences between bubaline and cattle milk are:

- Higher butirose fat (107% more).
- Lower cholesterol (17 to 24% less).
- Higher protein (34% more).
- Higher lactose (19% more).
- Higher total solid content (48% more).
- Lower salts (Na, Cl, K).
- Higher overall nutrient values.

Source [4].

Buffalo cow milk is defined by its nutrient profile; this facilitates its physico-chemical and organoleptic identification. It has higher viscosity, curding strength and pH [5]. It has characteristic sensory evaluation, slightly sweet flavor and bright white color due to almost complete carotene absence in its fat fraction [3]. Buffalo cow milk differences with its cattle counterpart are present also in the colostrum. Environmental factors such as climate conditions, nutrition, breed, management and lactation period influence milk composition. Due to its high casein content, buffalo cow milk is more difficult to digest; in India it is common to add 33% water when the milk is destined for human consumption [6].



COMPOSITION	BUFFALO COW MILK	COW MILK
Water	84,5	87,5
Dry extract	16-22	11-13
Lean extract	7-12	6-10
Casein	2,8-4,2	2,6-3,0
Fat	7,0-9,6	3,3-4,3
Lactose	4,9-5,0	4,6-5,2
Total protein	4,0-4,6	3,0-3,8
Ca (mg/100 g)	180-240	120
P (mg/100 g)	120-140	65
Ca/P	1.61	1.31
Ash	0,75-0,85	0,80-0,90
Acidity (°SH/50)	4,2-5,0	3,3-3,5
Density (15°C)	1.031-1.034	1.028-1.035
pH	6,6-6,8	6,6-6,7

Fig. (2). Comparative milk composition.

The comparative milk composition of cattle and buffalo are shown in Fig. (**2**) [7],

CHAPTER 11

Parasitological Scenario of Buffalo Farms in Central and Southern Italy: A Review

Antonio Bosco, Laura Rinaldi, Maria Paola Maurelli and **Giuseppe Cringoli**[*]

Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, CREMOPAR Regione Campania, Italy

Abstract: In this review, focus has been given on the diverse parasitological scenario that can be found in different buffalo farms in central and southern Italy, where the majority of buffalo heads are reared. The Geographical Information System, developed and used for the planning of sampling protocols, for data analysis and results, has been employed for the conduction of many studies performed in the course of the last twenty years. Furthermore, the copromicroscopic analyses were conducted using multivalent techniques, the FLOTAC techniques, which are characterized by high sensitivity, as well as precision, specificity, accuracy and reproducibility. Such techniques have been developed to further the capacity to quickly detect any possible parasitic infections, especially those that may pose any kind of human and veterinary public health concerns. From the results presented in this review, it is clear that we have witnessed over the last decades a significant modification of buffalo farm management and production system. Such buffalo farms have moved towards more intensive and innovative practices, such as the adoption of newly developed reproductive technologies and nutrition regimens together with increasing amounts of concentrate feeds and stored forages. The concurrent preventive use of anthelmintic treatments has greatly reduced helminth infections, which may pose a serious threat for human health such as the zoonosis caused by the larval stages of *Echinococcus granulosus*. It is worth mentioning also the witness of a parallel rise in protozoa and arthropoda infections.

---

[*] **Corresponding author Giuseppe Cringoli:** Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, CREMOPAR Regione Campania, Italy; Tel: +39 081 676799; Fax: +39 081 676569; E-mail: cringoli@unina.it

Giorgio A. Presicce (Ed.)

Keywords: Buffalo, FLOTAC, Italy, Parasites.

1. INTRODUCTION

The economy of many countries across most continents is heavily influenced by the powerful zoo-economy driven by the presence of buffaloes (*Bubalus bubalis*), belonging to both the river and the swamp sub species. In some countries, and especially in Italy, the buffalo may be linked directly to some peculiar and specific product, like the mozzarella cheese. The importance of this milk-derived product is so great, that, thanks to high quality of milk produced and transformed in mozzarella cheese, the introduction of a specific designation of protected origin (DPO), has been felt mandatory, and it is used nowadays across the entire Italian territory and used abroad to identify the original product. A number of countries are paying more and more attention to buffalo milk, its quality and, equally important, to its derived products, and this is reflected in the increased funding on various areas of research activity. In addition, the buffalo species is second only to cattle in the production of milk world-wide, accounting for a 12% of the totality of milk produced all over the world [1]. In consideration of the large interest expressed for this species, its zoo-economical implications and the health issues inevitably raised, a special focus addressed into the investigation of parasitic infection is of paramount importance. In fact, in tropical and sub-tropical countries, parasitic infections are very common and are unfortunately responsible for relevant economic losses in terms of dead animals following heavy parasitic infections, consequent disposal of infected organs at slaughter, and reduced rates of weight gain [2].

In addition, some of those parasitic infections are typically zoonosis as they can have a direct clinical relevance in the human species, in some cases according to the different geographical location, such as schistosomiasis in China and the Philippines, or other infections like cystic echinococcosis, cryptosporidiosis, fasciolosis and giardiasis which are reported everywhere. Currently, in buffalo farms spread over central and southern Italy, the parasitological situation has been strongly modified in recent decades due to, as anticipated earlier, the modernization of buffalo farms together with the use of modern reproductive strategies and nutritional technologies, and the introduction of prophylactic parasitic countermeasures, especially specific anthelmintic protocols and related treatments only following a clear and accurate diagnosis [3 - 5].

Considering that most buffaloes in Italy are reared in central and southern Italy, in this review an up-to-date summary of parasitism affecting this species in that part of Italy, from protozoa to helminths and arthropoda, will be given.

In Table **1**, a list of the various parasitic species affecting the buffaloes in central and southern Italy, is made available. The data reported in Table **1** came from a study performed in the Lazio Region (central Italy) on 127 farms and from diagnostic activities performed at CREMOPAR in the Campania Region (southern Italy) from 2000 to 2013 on 1,324 farms.

Table 1. Main parasitic infections in water buffaloes in central and southern Italy [3, 6 - 11].

Parasites	**Farm Prevalence (%) (min and max)**
Protozoa	
Eimeria spp.	94.5-97.7
Helminths	
Gastrointestinal strongyles	4.7-33.1
Strongyloides spp.	3.1-4.7
Fasciola hepatica	1.1-7.1
Dicrocoelium dendriticum	0.5-2.4
Paramphistomidae	2.3-7.1
Moniezia spp.	0.5-2.4
Echinococcus granulosus (larval stages)	8.0-12.4
Arthropoda	
Lice (*Haematopinus tuberculatus*)	2.8-11.0
Mange mites (*Psoroptes* spp.)	3.0-12.6

2. PROTOZOA

Infections by intestinal protozoa are a leading cause of neonatal diarrhoea, having a negative impact on the growth performance of buffalo calves.

In particular, buffalo calves are easily hit by coccidiosis, caused by several

CHAPTER 12

Folliculogenesis and Ovarian Physiology Applied to Reproductive Biotechnologies in Buffaloes

Pietro Sampaio Baruselli[1] and **Giorgio Antonio Presicce**[2,*]

[1] *Departamento de Reprodução Animal, FMVZ-USP, São Paulo, SP, Brazil*

[2] *ARSIAL, Regione Lazio, Via R. Lanciani, 38 - 00161 Rome, Italy*

Abstract: The review aims at illustrating the state of the art in terms of acquisition of knowledge in the reproductive physiology of the buffalo species, ranging from fetal oogenesis to prepuberal and adult follicular dynamics in river and swamp buffaloes. Such aspects are considered in parallel and compared to cattle reproductive physiology. Reproductive efficiency is presented in the light of a seasonal pattern, and affected by human intervention due to localistic differential market demands for milk and cheese production. Finally, the implementation of reproductive technologies, from the use of artificial insemination together with the development of protocols for synchronization of ovulation, to *in vivo* (MOET) and *in vitro* embryo production and Ovum Pick Up (OPU), are taken into consideration, highlighting successes and difficulties.

Keywords: Buffalo, Reproduction, Reproductive technologies.

1. INTRODUCTION

Buffalo has a great potential for milk and meat production, and in the last decade an increase of 39.1% and 15.3%, respectively, has been witnessed [1]. Despite most of the buffalo population is located in Asia (above 90%), its economical exploitation is also important in other continents (*i.e.* America and Europe). Reproductive biotechnologies have been used in this species in order to obtain a faster genetic improvement for the characters of interest. However, such newly developed strategies are not as efficiently implemented as in cattle. Some

---

[*] **Corresponding author Giorgio A. Presicce:** ARSIAL, Regione Lazio, Via R. Lanciani, 38 - 00161 Rome, Italy; Tel: +39 06 61597561; Fax: +39 06 61597561; E-mail: gpresicce@arsial.it

Giorgio A. Presicce (Ed.)

morphological and physiological features of buffalo reproduction could explain some of the encountered differences with the cattle counterpart and the results obtained up to now. This review focuses on the main biotechnologies applied to buffalo reproduction, emphasizing featuring aspects of reproductive physiology characterizing this species, which can affect the success of applied reproductive technologies.

2. FOLLICULOGENESIS

In the course of fetal development, germinal cells are produced following migration of primordial germ cells to the gonadal ridge. Upon colonization of the genital ridge, prophase of the first meiotic division occurs with a halt to further progression at the diplotene stage, until formation of primordial follicles [2]. Along with this progression, follicles go through a morphological modification of their anatomical structure. In the buffalo species, oogones are found within primordial follicles from 0 to 3 months of gestation, whereas preantral to antral follicles can be found between 4 to 6 months, increasing their number with the progression of gestation until its physiological end [3]. Typically, a significant decrease in the number of primordial to primary follicles is witnessed with increasing fetal age, and a concomitant increase in secondary follicles. There is a large individual variation in the number of follicles at different stages of development within different fetal ages [2].

A paucity of information is related to the number of germ cells in the buffalo species, although some authors have reported a 10-fold lower value (10,000 to 12,000) when compared to cattle [4, 5]. In addition, and typically in large ruminants, despite the thousands of available primordial follicles, the majority of them will become atretic and therefore not viable [2]. It has been reported that, in buffaloes, most follicles from ovaries collected at slaughterhouses at random stages of the reproductive cycle (92 to 95%) are estrogen inactive and atretic. The status of an atretic follicle is defined according to the molar ratio of E2 to P4 in the follicular fluid, and such atresia has been reported to be higher in small and medium rather than large follicles [5]. Le Van Ty *et al.*, also reported the existence of a lower number of antral follicles in buffalo, when compared to cattle (47.5 ± 23.8 *vs*. 233.0 ± 95.8; $P < 0.002$) [6]. Nevertheless, Presicce *et al.* [7], in prepuberal buffalo calves reported a high number of *in vivo* available antral follicles, similarly to what previously reported in cattle prepuberal calves [8]. A lower ovarian follicular pool, associated to a high level of atresia, can be accounted for the significantly lower number of antral follicles available within the follicular waves in adult buffaloes when compared to cattle. In a recent study [9], following a hormonally induced wave emergence in three different species, namely *Bubalus bubalis*, Bos indicus (Nelore) and Bos Taurus (Holstein) heifers, the mean number of antral follicles in buffaloes (13.1 ± 1.4) was similar to cattle (15.0 ± 2.8), but significantly lower than Nelore (29.7 ± 3.1).

3. ULTRASTRUCTURE OF PREANTRAL AND ANTRAL FOLLICLES

Adult buffalo ovaries have an average of less than 20 thousand preantral follicles [10]. The main modifications in the ultrastructure of preantral follicles begin with an enlargement from primordial to secondary follicles (35.0 ± 3.1 µm to 53.3 ± 12.0 µm, respectively), accomplished by an oocyte growth from 24.9 ± 3.7 µm to 29.4 ± 5.4 µm, respectively [11]. Additionally, there is an increase in the number of organelles, such as mitochondria (predominantly round-shaped), smooth and rough endoplasm reticulum, and also vesicles showing coalescence along this process. Until the primary stage, the zona pellucida cannot be seen (and after that, it is only partially developed in patches), and the oocyte and granulosa cells appear only juxtaposed. From this stage, oolemma projects into adjacent granulosa cells, which acquires a cubical form in secondary follicles [11].

With regard to antral follicles, there is a change in the organization of structures according to follicle development from 1 to 10 mm. The perivitelline space is initially formed and can be seen in 2 mm follicles and its growth is concluded in 10 mm follicles, when numerous oocyte microvilli can be found in it. The nucleus of oocyte is periferically located, and a well-developed smooth endoplasmic reticulum and Golgi apparatus can be seen in the perinuclear region. There is an increase in the proportion of pleomorphic mitochondria, instead of rounded-shape ones, reaching a peripherical location in the oolemma. Also, cortical granules (located in clusters) can be visualized in this area, increasing in number and size. Inversely, a medullar position is taken by lipid vacuoles, which increase in number and size, and coalesce as follicular growth occurs [11].

CHAPTER 13

Multiple Ovulation and Embryo Transfer in the Buffalo Species

Gianluca Neglia[*] and **Giovanna Bifulco**

Department of Veterinary Medicine and Animal Production, Federico II University, V. Delpino 1, 80137, Naples, Italy

Abstract: Multiple Ovulation and Embryo Transfer (MOET) is one of the biotechnologies of reproduction most utilized in the world to produce a high number of *in vivo* embryos. In the buffalo species the application of this technology meets several difficulties, and the embryo recovery rate is definitely lower than that recorded in cattle. This chapter aims at discussing the state of art of MOET in buffaloes and to analyze the factors that limit and influence its efficiency.

Keywords: Buffalo, Estrous cycle, *In vivo* produced embryos, Superovulation.

1. INTRODUCTION

The Multiple Ovulation and Embryo Transfer (MOET) technique or superovulation (SO) followed by embryo transfer (ET), was designed in order to modulate the oestrous cycle and achieve a large number of ovulations in species that are usually characterized by a single ovulation (Fig. **1**) [1]. The first results on this reproductive technology in cattle have been reported more than 50 years ago [2, 3]. However, despite the new findings in oestrous cycle manipulation and follicular development in this species, some aspects related to MOET are still unknown [4, 5]. In particular, the application of this technology in the field is limited by the variability in terms of response, and represents one of the main factors limiting a widespread use of this technology in the field. In any case,

---

[*] **Corresponding author Gianluca Neglia:** Department of Veterinary Medicine and Animal Production, Federico II University, V. Delpino 1, 80137, Naples, Italy; Tel: +39 081 2536063; Fax: +39 081 292981; E-mail: neglia@unina.it

Giorgio A. Presicce (Ed.)

MOET protocols in bovine, result on average in 5 to 6 transferable embryos/donor/treatment.

Fig. (1). Buffalo superovulated ovary.

MOET has also been applied in buffaloes. However, although the first successful embryo transfer in this species was performed in the United States of America in 1983 [6] and later in India [7], the application of MOET still results in low embryo yield when compared to cattle. In particular, following superovulatory treatment, buffaloes show good follicular response, moderate ovulatory rate and corpora lutea yield but, in contrast, low embryo recovery rate. Several attempts have been carried out in order to increase the number of recoverable embryos, but the causes for the low recovery rates are still unknown or at best speculative.

2. BASIC CONCEPTS ON SUPEROVULATION

The dynamics of follicular development occurring in waves during the oestrous cycle comes to help in achieving a better comprehension in the implementation of superovulation protocols in buffaloes. In some domestic species, like cattle and buffalo, a wave-like pattern is observed during the physiological estrous cycle [8]. Typically in cattle the estrous cycle is characterized by the development of 2 to 3 waves of follicular growth, although also cycles with 4 waves of follicle development have been observed [9].

Follicular cycles in the buffalo species are also organized in a wave-like pattern, in both natural [10] and synchronized [11] oestrus. In this species, more than 60% of animals show ordinarily two waves during the cycle and more than 30% shows three waves. The length of the cycle is typically 23-24 days, although shorter (16 days) and longer (26-30 days) cycles have been observed [11]. In buffaloes exhibiting a two-wave growth pattern, the first growth wave emerges on day 1 and the second on days 12 to 14. In those with a three-wave growth pattern, the emergence of the second and the third wave occurs on days 11 and 20, respectively [11]. During each wave, the phenomena of recruitment, selection and dominance occur [12]. During the recruitment, the growth of a cohort of 6-8 follicles is influenced by the increasing circulating levels of FSH [10]. Subsequently, the recruited follicles undergo selection and dominance processes: in the buffalo, being a monovular species, normally a single follicle develops into a dominant follicle, while the remaining follicles regress.

The phenomenon of dominance occurs when the dominant follicle reaches the diameter of approximately 7.4 mm, after 3 days of growth [13]: this phase is recognized as deviation, when the follicle acquires the receptors for LH. Interestingly, the capability of becoming a dominant follicle is retained by all growing follicles before deviation occurrence, whereas once deviation has occurred, only the largest follicle develops into a dominant follicle and the others undergo regression. It is known that during the phase of dominance, the LH plays a key role, since some studies carried out in bovine heifers demonstrated that LH receptors are expressed in granulosa cells of the future dominant follicle about 8 hours before deviation [12]. Furthermore, the intrafollicular estradiol: progesterone ratio declines, preparing the ovulation [14].

As introduced above, the aim of superovulation is to allow more ovulations within the estrous cycle of the animal. Therefore, the follicular dynamics is altered and modulated by the exogenous administration of hormones several days in advance of the anticipated normal estrus in an effort to elicit multiple ovulations in the donor animal. This is inevitably obtained by overriding the phase of dominance,

CHAPTER 14

Applied Reproductive Technologies in the Buffalo Species

Bianca Gasparrini[*]

Department of Veterinary Medicine and Animal Production, Federico II University, Via F. Delpino 1, 80137 Naples, Italy

Abstract: In consideration of the typically low efficiency of MOET programs in buffaloes, in the past two decades there has been a mushrooming interest in the exploitation of *in vitro* embryo production procedures (IVEP), employed for a more rapid and targeted improvement and propagation of superior genetics from elite animals. Procedures that had been used in cattle successfully, were also used in the very first attempts in buffaloes, although a significant improvement in the efficiency of the entire process in the buffalo species has been achieved in the course of the intervening years through novel information on oocyte and embryo culture requirements. This review aims at describing the state of the art of IVEP in the buffalo species, the results and improvements obtained together with the difficulties and limitations still to be overcome.

Keywords: Buffalo, Cryopreservation, *In vitro* produced embryos, Sperm sexing.

1. INTRODUCTION

Buffaloes have been playing over time an important role in countries characterized by a number of disadvantages, both in terms of climate and agricultural production systems. Interest in this species has then, for these main reasons, grown up steadily in recent years. The river buffalo can be considered a dairy producer that has no match in developing countries, characterized also by

---

[*] **Corresponding author Bianca Gasparrini:** Department of Veterinary Medicine and Animal Production, Federico II University, *Via* F. Delpino 1, 80137 Naples, Italy; Tel: +39 081 2536284/461; Fax: +39 081 292981; E-mail: bgasparr@unina.it

Giorgio A. Presicce (Ed.)

particular environmental conditions typical of the tropics north of the equator. In those climate conditions, the buffalo is used to compensate for the lack of cattle milk in the course of the rainy season (winter to spring), enabling thus people to make use of animal proteins at competitive costs. This can happen thanks especially to the special interaction occurring between reproductive seasonality typical of this species, together with availability of forage throughout the year and external environmental conditions. A striking evidence underlining the importance of this species is given by the mushrooming increase in the total world buffalo population in the course of the last 40 years, adding up to 86% especially when compared to the cattle counterpart, with only a 34%. In Italy the Mediterranean Italian buffalo is the only livestock species that has shown a growing trend over the years; it is an important economic resource, due to the high market demand for mozzarella cheese, and its genetics is highly requested around the world due to its high milk production. The increasing demand for buffalo milk and the need to cut production costs make genetic improvement critical for successful buffalo breeding. In this scenario, programs aimed at selecting the best animals through the adoption of newly developed reproductive technologies, can be completed in shorter times than usually expected under ordinary reproductive management. In addition, developing countries require the availability of high genetic animals as quickly as possible, and in this context, reproductive biotechnologies may offer a sharp help for example in replacing the working efficiency of local swamp buffaloes with a much higher efficiency in terms of milk produced from river buffaloes. This is a possible way to enhance the availability of animal proteins for human need and consumption. In the past, one of the most applied reproductive technology in cattle, was also employed in buffaloes, in order to increase and speed up the genetic gain *via* the maternal lineage. In fact, attempts were made to induce multiple ovulations for *in vivo* embryo production in buffalo (MOET programs), although the obtained results have always been inconsistent and embryo production was always significantly reduced when compared to the cattle counterpart [1 - 3]. For this reason, and thanks to the encouraging results reported in cattle, interest has been shifting to the possibility to produce embryo following use of *in vitro* procedures (IVEP).

The first IVEP experiences were carried out on abattoir-derived oocytes mainly for research purposes and for the genetic rescue of highly valuable animals with terminal illness or characterized by reproductive inefficiency, or else for mass production of embryos. Very little impact on the genetic enhancement of animals is exerted by the use of IVEP procedures for embryo mass production, using oocytes derived from ovaries of slaughtered animals.

A boost in the spreading of IVEP procedure for the improvement of genetically important traits in production animals, has been seen thanks to the development of techniques for recovering immature oocytes from antral follicles in live animals, termed Ovum Pick Up (OPU). Indeed, the *in vivo* collection of immature oocytes by transvaginal ultrasound guided follicular aspiration, provided the lacking link between IVEP technologies and animal breeding. The genetic progress through the maternal lineage can be more efficiently and more quickly achieved through the combined use of IVEP procedure and OPU technique, thanks also to the repeatability of the procedure and its non invasive approach, leading to a higher number of embryos produced on a long term basis. In addition to the poor embryo output of MOET [1 - 3], and in comparison to MOET programs, characterized in buffaloes by a low efficiency in terms of embryos produced and recovered [1 - 3], the synergistic coupling of IVEP and OPU allows a consistent and wider production of embryos from animals that would have been otherwise discarded, such as in the case of acyclic females, reproductive failures due to salpingitis or infections of the genital tract, unresponsiveness to hormonal administration for superovulation. In addition, even pregnant animals within their first trimester can be used for embryo production through OPU.

The introduction of OPU in cattle dates back to the late 1980s, and became operational in the early 1990s. OPU was first adopted in 1994, in buffaloes found in deep anestrus characterized by hypotrophic ovaries [4, 5], and since then it has spread in many other countries other than Italy [6 - 13], like Brazil [14, 15], China ([16, 17], Argentina [18] and India [19, 20]. Therefore, in light of some of the above mentioned aspects, the coupling of IVEP and OPU techniques is the most productive and efficient approach for elite embryo production (transferable embryos) on an animal-donor basis and over long period of times. In addition, and especially in the buffalo species, this is a very competitive combined technology in terms of embryo yield when compared to MOET programs, due to the fact that

CHAPTER 15

Buffalo Cloning and Transgenesis

Gautam Kumar Deb[1,4], **Rangsun Parnpai**[3] and **Il Keun Kong**[1,2,*]

[1] *Division of Applied Life Science (BK21plus Program), Graduate School of Gyeongsang National University, Jinju, Gyeongsangnam-do, Republic of Korea*

[2] *Institute of Agriculture and Life Sciences, Jinju 660-701, Gyeongnam, Republic of Korea*

[3] *Embryo Technology and Stem Cell Research Center, School of Biotechnology, Suranaree University of Technology, Nakhon Ratchasima 30000, Thailand*

[4] *Bangladesh Livestock Research Institute, Savar, Dhaka–1341, Bangladesh*

Abstract: Somatic cell nuclear transfer (SCNT) is a relatively new technique applied to the buffalo species. The current oocyte maturation, enucleation and nuclear transfer, and *in vitro* embryo culture systems for buffalo reconstructed embryos, are comparable with bovine SCNT. However, there are very few reports on delivery of normal live cloned buffalo calves. The inducible pluripotent stem cells (iPS), which made a major breakthrough in the remodeling of somatic cell nucleus by inducing different transcription factors, have not been applied in the buffalo cloning. Moreover, the application of SCNT in transgenesis of buffalo is limited to the development of transgenic blastocysts. In this chapter, we described recent advances and novel approaches in SCNT and transgenic buffalo productions in connection with the concurrent advances in other relevant mammalian species.

Keywords: Buffalo, Cloning, Induced pluripotent stem cells, Somatic cell nuclear transfer, Transgenesis.

1. INTRODUCTION

Buffaloes (*Bubalus bubalis*) are characterized by a series of physiological fea-

---

[*] **Corresponding author Il Keun Kong:** Division of Applied Life Science (BK21plus Program), Graduate School of Gyeongsang National University, Institute of Agriculture and Life Sciences, Jinju 660-701, Gyeongnam, Republic of Korea; Tel: +82 055 772 1942 and +82 010 3785 3567; Fax: +82 055 772 1949; E-mail: ikong@gnu.kr.ac

Giorgio A. Presicce (Ed.)

tures such as late onset of puberty, silent estrus, seasonal anestrus, long post partum anestrus and long calving interval [1]. Moreover, the ovaries of buffaloes present lower numbers of follicles, leading to few oocytes available for *in vitro* embryo manipulation. Consequently, progress in the application of assisted reproductive technologies has been slow in this species [2]. The first cloned buffalo calves were born in 2007 [3], approximately 16 years after the first buffalo calf born from *in vitro* fertilization [4]; however, there is no report of a live transgenic buffalo clone to date.

When any organism is genetically identical to another, it is considered to be a biological clone. Cloning is a complex process leading the to the production of an identical genetic organism through the procedure of somatic cell nuclear transfer (SCNT), as defined by the American Medical Association. Often though, the term cloning is also used, in a broader definition, to include the production of tissues and organs from cells or tissue cultures adopting stem cells. The first cloning experiment in farm animals was conducted in the late 1980s to produce monozygotic twins by cutting an embryo into two halves and each half then transferred in an empty zona pellucida in sheep [5] and cows [6]. A tremendous advance in farm animal cloning was reached with the birth of Dolly the sheep in 1996, by using the SCNT procedure [7]. Dolly was produced by transferring an adult somatic cell nucleus into an enucleated oocyte. Since then, clones have been born in different farm animal species using various somatic cell nuclei derived from fetuses to adult animals. The birth of Polly was considered the second landmark in biology and farm animal cloning, as this animals was a genetically modified clone, which opened a new horizon in farm animal cloning [8]. Transgenic farm animals, which are a group of animals which have been genetically modified, are characterized by having inserted in their genome, one or more genes derived from another organism, and following the birth of Polly, more farm animals have been cloned by SCNT such as goat [9], pig [10 - 12] and cattle [13 - 15].

Recently, the reprogramming of somatic cells in animals has been achieved through the forced induction of several transcription factors. The reprogrammed cells present the properties of embryonic stem cells and are known as induced pluripotent stem cells (iPS) [16]. IPS cells are able to produce live mouse chimeras [17 - 19]. Why such excitement in a successful cloning? In breeding programs, in order to quicken the disseminations of genes from superior genetic animals to less valuable genetic animals within commercial populations for generating transgenic animals, cloning is instrumental due to its intrinsic features of increasing the accuracy of selection and the genetic progress. Unfortunately, the main limitation of this novel strategy, is the low success rate of the overall process and consequently the high cost per single transgenic animal produced to be ready for reproduction [20]. In this chapter, the authors describe the current state of cloning and transgenesis in the buffalo species.

2. SOMATIC CELL NUCLEAR TRANSFER

By SCNT is meant a laboratory procedure performed in order to de-differentiate a somatic cell, like a fibroblast or any other adult somatic cell, so that a nuclear reprogramming can occur when subsequently, the same somatic cell is inserted into an enucleated recipient oocyte [21]. By employing such technical procedure, a number of genetically identical animals can be obtained. Nuclear transfer is a powerful tool for studying genomic imprinting, nuclear-cytoplasmic interaction, totipotency, and the contribution of paternal and maternal genomes to developing embryos. However, it is an inefficient method for cloning farm animals, achieving 0 to 4% live births [22]. Parameters affecting the success rate include the age of donor cells, cell cycle stage, ploidy and developmental stage of recipient cells [23], and activation schedule following fusion [22]. The SCNT procedure includes several steps as described below (Fig. **1**).

2.1. Oocyte Recovery

Three methods are widely used for retrieving immature oocytes from abattoir ovaries in buffalos (Table **1**): 1) slicing, 2) follicle puncture, and 3) follicle aspiration. The follicular aspiration method is commonly used for the retrieval of COCs for SCNT and transgenic operations in buffalos. The mean recovery of good quality oocytes per abattoir ovary is between 0.4 to 2.4 [24 - 28], which is lower than cattle (8–12 good quality oocytes/abattoir-derived ovary). Ultrasound-guided ovum pick (OPU) from live animals slightly increases the number of oocytes retrieved [29 - 31]. The buffalo ovary is smaller in size [32] and contains

SUBJECT INDEX

Giorgio A. Presicce (Ed.)

GIORGIO ANTONIO PRESICCE

He is currently employed by a governmental regional agency, dealing with animal production and focussing on the application of reproductive technologies for the improvement of production traits in farm animals. He has served for FAO in The Gambia at the International Trypanotolerant Center. He received his doctoral degree and post-doctoral experience at the Department of Animal Science, Cornell University, USA in farm animal reproductive physiology and embryo biotechnologies, with emphasis on in vivo and in vitro embryo production, Ovum Pick Up, parthenogenetic development and cloning. He was a visiting Assistant Professor at the same Department of Animal Science, Cornell University, and Senior Researcher at PPL Therapeutics in Blacksburg (VA) in charge of reproductive technologies of the transgenic cattle herd. He has consultancies with the following Institutions: a) University of Connecticut (USA) – Department of Animal Science in Storrs (Prof. Xiangzhong Yang); b) China Agricultural University – National Laboratories for Agrobiotechnologies, in Beijing (Prof. Li Ning); c) Kunming Institute of Zoology in Kunming (Prof. Weizhi Ji); d) Guangxi Buffalo Research Institute in Nanning (Prof. Yang Bingzhuang); e) Philippine Carabao Center – City of Munoz, The Philippines (Dr. Libertado Cruz); f) Gyeongsang National University, Institute of Agriculture and Life Sciences, Republic of Korea (Prof. Keun Kong); g) Department of Physiology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand (Prof. Mongkol Techakumphu); h) Department of Animal Physiology, Hue University of Agriculture and Forestry, Vietnam (Prof. Vo Thi Kim Thanh). He has been in scientific collaboration established with Dr. Fuliang Du, President and co-founder of Renova Life, Inc. He has been invited speaker in the national and international meetings, with over 60 publications in the peer-reviewed international journals. He was a recipient, in 2006, of the "China Friendship Award" for the scientific contribution and introduction of newly developed reproductive technologies within new strategies for the improvement of genetic gain in water buffaloes. Selected bibliography: 1) An et al., (2016). Successful Vitrification of In vivo Embryos Collected from Superovulated Japanese Black Cattle (Wagyu). Reprod Dom Anim; 2) An et al., (2015) Efficient cryopreservation of mouse embryos by modified droplet vitrification (MDV). Cryobiology; 3) Campanile et al., (2013). Effect of Season, Late Embryonic Mortality and Progesterone Production on Pregnancy Rates in Pluriparous Buffaloes (Bubalus bubalis) Following Artificial Insemination with Sexed Semen. Theriogenology; 4) Senatore et al., (2010). Improved in vitro Development of OPU Derived Bovine (Bos taurus) Embryos by Group Culture with Helper Embryos Embedded in Agarose Chips. Theriogenology; 5) Presicce (2007). Reproduction in the water buffalo. Reprod Dom Anim; 6) Presicce et al., (2005). First established pregnancies in Mediterranean Italian buffaloes (Bubalus bubalis) following deposition of sexed spermatozoa near the utero tubal junction. Reprod Dom Anim.