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ScienceDirect
Theriogenology 68 (2007) 454–460
Theriogenology
www.theriojournal.com

Importation of in vitro-produced *Bubalus bubalis* embryos from Italy into the United States: A case report

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Abstract

On December 19, 2005, 14 in vitro-fertilized water buffalo (*Bubalus bubalis*) embryos, which had been cryopreserved by vitrification, were thawed and transferred into *B. bubalis* recipients in California. The embryos had been produced in Italy, following transvaginal oocyte pickup (TVOPU), with subsequent in vitro maturation, insemination, and culture. This case study relates our experience in meeting the regulatory criteria, established by the Animal Import/Export Office of the USDA-Animal and Plant Health Inspection Service (USDA-APHIS), in order to successfully import these embryos into the USA.

Keywords: *Bubalus bubalis*; Importation; USDA-APHIS-VS; IVP embryos; Regulations

1. Introduction

From the earliest days of the commercial bovine ET industry in the 1970s, ET promised to be a means of introducing new germ lines across international boundaries [1,2]. Concurrently, leaders in the ET field acknowledged the potential risk of simultaneously introducing foreign animal diseases [1–4]. Advances that have brought about a partial realization of the potential for international movement include improvements in diagnostic sensitivity and specificity of tests applied to potential donors [5]. In addition, improvements in cryopreservation of embryos, standardization of hygienic procedures for embryo handling, and research that has documented the general safety of international ET, have all contributed to international movement of embryos [6–12].

The agency in the USA responsible for overseeing import and export of animals and animal products (including embryos) is the Animal Import/Export Office of the USDA-Animal and Plant Health Inspection Service (USDA-APHIS). All permits for importation of embryos and semen must be issued by this office. This paper discusses the major criteria which had to be met in order to import ''new germ lines'' of water buffalo (*Bubalus bubalis*) into the USA from Italy.

2. Signalment and history

A private entrepreneur with an interest in establishing a *B. bubalis* dairy industry in California contacted

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0093-691X/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.theriogenology.2007.05.049

the School of Veterinary Medicine, University of California at Davis. His first request was for assistance with importation of live water buffalo from the Caserta region of Italy. When told that such importation would be impossible (see Section 2.1), he decided to pursue a program of embryo importation, with the goal of producing highly selected Italian Mediterranean buffalo, and breeding this ''founder group'' with imported semen from progeny-tested bulls in Italy.

2.1. Obstacles to importation

2.1.1. Live animal importation

Following the European outbreaks of Bovine Spongiform Encephalopathy (BSE) in the 1980s and Foot and Mouth Disease (FMD) in 2001–2002, importation of live ruminants was essentially prohibited, with the exception that Mexican and Canadian cattle could be brought into the USA for fattening and/or slaughter. The Harry S. Truman animal quarantine facility (close to the coast of Florida) was deactivated, and in any case, had not been fully utilized because of the high expense importers had to bear in order to keep animals at this facility [13]. As an example, importation of 500 Boer goats from South Africa was estimated to cost $2000 per goat ($1M total) for the quarantine alone, excluding all testing costs. Importers received permits on a lottery system, and were required to submit a cashier's check for $32,000 for each application to the lottery [13].

2.1.2. Other obstacles

Acute animal disease situations in many countries may prohibit the importation of bovids and bovid products into the USA. In addition, non-tariff trade barriers, e.g., where technical or political issues rather than scientifically derived policies make it difficult or impossible to import/export the product in question, can also be obstacles.

2.2. Consortium formation

An informal consortium was formed among the client and faculty members of the veterinary colleges and schools at the University of California, Davis, the University of Florida, and Federico II University (Naples). The roles of the consortium members were as follows: Italian scientists: (a) procurement of permission from Italian buffalo dairymen to collect oocytes from their animals; transvaginal ovum pick-up; IVF procedures; vitrification of blastocysts; procurement of official paperwork from appropriate Italian government agencies [14]; (b) UC Davis and University of Florida scientists: selection of domestic recipients; procurement of embryo import permit from USDA-APHIS; receipt of transported embryos; transfer of embryos to recipients; (c) client: funding of importation, animal purchase and maintenance, and payments to Italian dairymen.

2.3. Importation considerations

2.3.1. Practical considerations—availability of recipients

The exact number of *B. bubalis* in the US is unknown, but probably amounts to a few thousand head, mostly descendants of two importation waves in the late 1970s (one from Guam and one from Trinidad-Tobago [15]). The buffalo from Guam were swamp types (karyotypes $2n = 48$), whereas those from Trinidad-Tobago were so called ''buffalypso'' breed, a composite of river type breeds ($2n = 50$) [15,16]. The distribution of karyotypes amongst the current US population is also unknown, but includes river-swamp hybrids ($2n = 49$), which are reported to be fertile and able to carry embryos of either swamp or river buffalo [16]. The largest concentration of potential recipient buffaloes was a Florida herd of >1000 animals. From this farm, the client negotiated direct purchase of 44 animals meeting the following criteria: (a) manifest fertility (i.e. calf at side); (b) involuted and normal reproductive tract (on the basis of transrectal palpation); (c) a cervix that would allow the passage of an ET pipette; and (d) dairy character, as evidenced by udder conformation, frame type, and to a limited extent, by disposition.

2.3.2. USDA-APHIS requirements, and resources for meeting them

The import protocols of the USDA APHIS animal import-export office are based on recommendations from World Organization for Animal Health (OIE), and from the International Embryo Transfer Society (IETS), as well as on comments from the public. Excellent sources for information regarding the disease status of all exporting countries can be found in: the OIE Terrestrial Animal Health Code, which is updated frequently [17]; the OIE World Animal Health Reports, which details animal health status and disease incidence for so-called ''A list'' and ''B list'' diseases, country by country [18]; and a quarterly OIE Bulletin, which is published on-line and is available at no charge [19].