Boosting Asia's buffalo population

Artificial reproductive biotechnologies for buffaloes

Buffalo has remained an important part of the Asian rural economy as a source of meat, milk and draft. However, the considerable decline in buffalo population has become a matter of serious concern, and artificial reproductive biotechnology holds great potential to reverse this trend.

BUFFALO HAS BEEN AN INTEGRAL PART of livestock agriculture in Asia for over 500 years now, producing draft power, milk, meat and hides. There are about 174 million buffaloes in the world (FAO Statistics), and roughly 97 percent or 167 million heads are found in the Asian region. However, despite the importance of buffalo to the economic and social fabric of the region, its population has been declining, partly due to poor reproductive performance. Artificial reproductive biotechnology is one of the key technologies to reversing this trend.

There are two major types of buffalo: the swamp buffaloes which can be found in most Southeast Asian countries; and the river buffaloes which are found more in India and Pakistan. The river buffaloes, also called the milking buffalo, produce considerable amounts of milk, allowing the Indian dairy industry to contribute 52 percent of the world production of buffalo milk.

There are many reasons for the decline of buffalo populations, foremost of which are: increased agricultural mechanization; increased urbanization, industrialization, and reforestation limiting paddy areas for buffaloes; growing buffalo slaughter rate to satisfy meat demands of a fast-growing population; poor reproductive performance; and lack of proper attention by policy makers and researchers.

The low reproductive efficiency in female buffalo can be attributed to delayed puberty, higher age at calving, long postpartum anoestrus period, long calving interval, lack of overt sign of heat, and low conception rate. In addition, female buffaloes have few primordial follicles and a high rate of follicular atresia.

Buffalo can adapt to harsh environments and live on low quality forage. However, its reproductive efficiency is often compromised by such conditions. Climatic stress depresses ovarian cyclicity, estrous expression, and conception rates. Poor nutrition usually related to seasonal fluctuations in availability and quality of feed, delays puberty and increases the duration of postpartum anoestrus. Management factors such as the system of grazing (free, tethered, or none) and sucking by calves (restricted or ad libitum) also alter

Photo courtesy of S.N. Lee, Livestock Research Institute, COA, Taiwan ROC.

Spotted buffalo at the Indonesian Institute for Science.

Laboratory demonstration of buffalo sperming during the workshop.

reproductive functions. Lack of selected bulls in some regions also affects buffalo reproduction.

The biotechnology workshop

Biotechnology can accelerate the improvement of animal production through the introduction of desirable traits (e.g. higher milk yield, or better growth rate), while conventional breeding takes decades to produce major changes.

The international seminar on artificial reproductive biotechnologies for buffaloes primarily aimed to serve as a venue for the exchange in expertise and reproductive technologies on buffalo, as well as the introduction of techniques on somatic cell cloning in dairy cattle. During the seminar, the status and challenges in buffalo production in each country in Asia, as well as its economic and social benefits, were shared and discussed. The activity has contributed much to a better understanding and appreciation of reproductive biotechnology techniques for buffalo, toward enhancing the dissemination and application of the technology in each participating country.

Reproductive biotechnology for buffaloes

The technical presentations during the workshop considerably covered the use of artificial breeding techniques such as artificial insemination (AI), estrus synchronization, embryo transfer, embryo freezing, *in vivo* maturation (IVM), *in vitro* fertilization (IVF), and somatic cell cloning for buffalo. These techniques, which have been used in a limited way in countries where intensive management of buffalo is practiced, allow the distribution of elite buffalo genes and the reduction in generation interval, and provide continued genetic gain and increased production of buffalo meat and milk.

One of the most important areas in improving female reproduction is the ovarian follicular dynamics in buffalo species, the knowledge of which may lead to better synchronization and superovulation protocols as well as embryo production and transfer. Obtaining higher pregnancies from artificial insemination (AI) through appropriate timing within the follicular wave, getting higher yield of eggs or embryos through super-stimulation at appropriate time of the wave, or improving harvest of oocyte for IVM/IVF through ultrasound guided transvaginal (Ovum Pick Up or OPU), are interesting developments and tools in improving livestock production efficiencies.

Artificial insemination (AI). The AI procedure has played a valuable role in facilitating appropriate genetic improvement in animal populations, through widespread use of outstanding males and dissemination of superior genetic material. The offspring will carry 50 percent of male genetic trait. AI can accelerate the introduction of new genetic material by importing frozen semen rather than live animal thus, reducing transport cost. The success of this procedure depends on proper freezing technique, estrus detection, animal management, and nutrition.

Superovulation. Superovulation is done on genetically superior female treated with hormones to induce simultaneous egg production. These eggs can be fertilized with the sperm through in vivo or in vitro methods, and the embryo produced can then be implanted into surrogate mothers (recipients). The donor (superovulated female) should be

Demonstration of spermatozoa sexing manipulation in water buffalo.

selected for genetic trait, in the case of buffalo, for milk and meat production and draft power.

In vivo embryo production (IVEP). The technique of IVEP involves multiple ovulation of elite donor, breeding (natural or AI) with top sires, and subsequent embryo collection, embryo conservation, and transfer to estrus/ovulation synchronized surrogate mothers. The serious limitation of IVEP is poor superovulatory response of nearly half of the donors following superovulation treatment. Follicle pool in buffalo is low and a high rate of follicle atresia may contribute to the low outcome of superovulation.

In vitro fertilization (IVF). It is now possible to adopt a fully in vitro system, whereby the immature oocytes are matured through in vitro maturation technique, followed by fertilization with in vitro capacitated spermatozoa and culture of newly formed zygotes in suitable media for development up to the transferable stage. IVF is the most economic and efficient method in large quantity embryo production.

Cryopreservation of buffalo genetic resource. The cryopreservation technique serves as an addition to other existing reproductive biotechnologies in buffalo. In 2001, successful production of buffalo calves after the transfer of vitrified buffalo embryos has been reported. However, cryopreservation of buffalo oocytes by slow freezing procedure was generally met with limited success due to its inherent sensitivity to chilling. Vitrification is an alternative step to freeze oocytes.

Embryo collection. The optimum time for nonsurgical embryo collection is based on the transport rate of embryos in superovulated mothers. Embryo development from superovulated buffalo varies considerably and various stages of embryo may be recovered from the same donor.

Somatic cell nucleus transfer (SNT). Cloning holds the promise of bypassing conventional breeding procedures to allow creation of thousands of precise duplicates of genetically engineered animals in a single generation. Factors affecting the efficiency of cloning in buffalo species involving activation procedures are the age and type of donor cell, the stage of donor cell cycle, and nuclear programming following nuclear transfer. Nowadays, cloning is an efficient technique for producing copies of elite livestock and transgenic animals. However, the overall efficiency of cloning is typically lower than 10 percent, represented by the number of live offspring as a percentage of the number of nuclear transferred embryos. High incidence of developmental abnormalities in cloned animal and pregnancy loss has also been encountered.

Future prospects

In the past two decades, some developing countries in Asia have considerably invested on embryo biotechnology laboratories and skilled manpower toward adopting the technology as an important tool for the faster multiplication of elite buffaloes and their genetic improvement. Conventional in vivo embryo production technology can be effectively used for the production of breeding stock of buffaloes. However, much research still needs to be done in terms of improving the efficiency of embryo transfer, especially in respect to superovulatory response, embryo recovery, embryo freezing, and conception following embryo transfer. Despite some

encouraging results, more studies and investigations are required to improve the efficiency of in vitro embryo and calf production so that the same could be used for research in the areas of sexing, cloning, transgenesis, stem cell techniques, and in the overall breeding programs for the genetic improvement of buffalo.

International Seminar on Artificial Reproductive Biotechnology for Buffaloes

Held in RIAP, Bogor, Indonesia on August 29 - September 1
No. of countries participating: 8 (Cambodia, India, Indonesia, Malaysia, Philippines, Taiwan ROC, Thailand, Vietnam)
No. of papers presented: 18
No. of participants: 60
Co-sponsor: Research Institute for Animal Production (RIAP), Indonesia

List of papers

Resource papers

1. Ovarian follicular dynamics in water buffalo (*Bubalus bubalis*) and its exploitation towards better ovarian responses
   - Libertado C. Cruz, Philippine Carabao Center (PCC), Philippines
2. Recent advances in somatic cell nuclear transfer and transgenic in cattle and goat in Taiwan
   - Shan-Nan Lee, Livestock Research Institute (LRI), COA, Taiwan ROC
3. Superovulation in buffalo in Indonesia
   - Polmer Situmorang, RIAP, Indonesia
4. Application of embryo biotechnology to augment reproduction and production in buffaloes: current status and future possibilities
   - A.K. Misra, ICAR, India
5. Role of reactive oxygen species in the buffalo sperm fertility assessment
   - Kanchana Markvichitr, Kasetsart University, Thailand

Country papers

6. Situation of buffalo raising in small-holder farms in Cambodia
   - Bun Tean, Royal University of Agriculture, Cambodia
7. Research and application of advanced reproductive biotechnologies in buffalo in Vietnam
   - Nguyen Huu Duc, VAST, Vietnam
8. Somatic cell cloning in swamp buffalo (*Bubalus bubalis*)
   - Jumnian Saikhuna, Mahidol University, Thailand
9. Conservation of water buffalo in Taiwan: cryopreservation of spermatozoa
   - Lin Yung Wei, Hualien Animal Propagation Station, LRI- COA, Taiwan ROC
10. Buffaloes in Indonesia
    - Endang Triwulanningsih and Lisa Praharani, RIAP, Indonesia
11. Physiology and biotechnology in swamp buffalo (*Bubalus bubalis*) reproduction
    - Mongkol Techakumphu, Chulalonkorn University, Thailand
12. Current status and challenges in buffalo production in Malaysia
    - O. Abas Mazni, MARDI, Malaysia
13. Recent advances in embryo transfer of swamp buffalo in Taiwan
    - Shiao Tzong Faa, LRI-COA, Taiwan ROC
14. Buffalo in Vietnam: Status and some applied reproductive biotechnologies
    - Chung Anh Dung, Institute of Agricultural Sciences for Southern Vietnam, Vietnam
15. Buffalo production scenario in India: opportunities and challenges
    - O.P. Dhanda, ICAR, India
16. Reproductive biotechnology in water buffaloes in the Philippines: recent developments and future perspectives
    - Eufrocina P. Atabay, PCC, Philippines
17. Current attempt to interspecies cloning in Taiwan
    - Ting Yung Kuo, LRI-COA, Taiwan ROC
18. Potentials and application of reproduction technologies of water buffaloes in Indonesia
    - J.T. Batosamma, Hasanuddin University, Indonesia

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