The role of biotechnology in animal production

S Said, P P Agung, W P B Putra and E M Kaiin
Research Center for Biotechnology, Indonesian Institute of Sciences, Jl. Raya Bogor Km. 46 Cibinong 16911, West Java, Indonesia

Email: syahruddinsaid01@gmail.com

Abstract. Biotechnology in animal production is widely used to increase not only the number of population of livestock animals to meet the requirement for world demand of animal products but also for endangered species to enhance the propagation and sustaining the current levels of biodiversity and genetic diversity. Biotechnologies can affect efficiency of reproduction and therefore also selection programs (artificial insemination, embryo transfer, sexing, cloning and other related techniques), improve determination of genetic values of animal (genetic markers, candidate genes and other related techniques), and biotechnologies can transform artificially the genome at the DNA level such as genetic engineering, gene transfer, and related techniques. The objective of this study was to review the importance of the most common current biotechnologies on animal production and some results that have been implemented in Indonesia.

1. Introduction
In general, biotechnology is the application of scientific and engineering principles that use living organisms or substances from these organisms to make or modify products, enhance plants or animals, or develop microorganisms for specific uses that are beneficial to humans [1,2]. It ranges from the conventional biotechnology also called traditional biotechnology is the application of biotechnology with a simple process such as baking, making use of yeast to the complex, the recombinant DNA technology or the classical to include biofertilizers, biological nitrogen fixation and fermentation to the modern biotechnology which ranges from plant tissue culture, cell culture, recombinant diagnostic and genetic engineering [2,3]. Now day’s biotechnology is mostly identified with applications in medicine and agriculture based on the knowledge of the genetic code of life. One of the benefits of biotechnology in animal husbandry is being able to provide increased livestock productivity.

Livestock production is one of the quick-growing agricultural sectors in developing countries, where it calculates more than a third of agricultural GDP. Many developing and transition countries have actualized high economic growth in recent years. This, combined with an increasing population and urban population and growth in personal incomes, is change the lifestyle and purchasing patterns with respect to food products. Global food protein demand is moving from plant proteins to animal proteins. It is projected that the demand for animal products will almost double by 2030 and that a great proportion of this increase will be in developing countries and from monogastric animals [4].

This growing demand for livestock products termed the “Livestock Revolution”, is produce a chance for increasing the welfare of millions of poor people who hinge on livestock for their livelihoods and could become a key means of reducing poverty. It has been reported that the world human consumption for the animal protein was 29 g per capita daily. Especially in the advanced countries, human consumption was approximately 90 kg of animal protein per capita consumption. However, the tendency
toward increased per capita demand for animal source foods is occurring usually in developing countries (80% of world population) [5]. According to Indonesia's situation, the average meat consumption is 2.72 kg per capita per year and is prospective to increase to 3.36 kg per capita per year by 2020 [6], this consumption is very low compared to world consumption.

By using biotechnology, it has possible to improve the productivity of animals via increase growth, quality of carcass and reproduction, improved nutrition and feed utilization, increased quality and food safety, improved health and welfare of animals and reduced waste through more efficient utilization of resources. Therefore, the biotechnology of livestock production is growing faster than in any other sector. By 2020 livestock is forecast to become the most important agricultural sector in terms of value-added commodity [5]. This paper reviews the importance of the most popular current biotechnologies on animal production and some results that have been implemented in Indonesia.

2. Animal biotechnology production in developing countries

The current developments in biotechnology have expose up stimulating possibilities for a rapid improvement in the productivity of domestic animals through its applications. Biotechnology of animal can help to improve in animal productivity in various ways namely by improving the production of products, by promoting growth and improving nutrient intake efficiency, by increasing the rate of reproduction of domestic animals, and by increasing the quality of animal production [7].

There are a large number of technologies that have been developed to the livestock, the main technologies that are applied effectively in livestock production in the developing country include conserving resources of animal genetic, increasing reproduction, embryo transfer (ET) and related technologies.

2.1. Reproductive biotechnology

The objectives of using reproductive biotechnologies in livestock are to increase production, improve reproductive efficiency and rates of genetic improvement. Over the years, many options have made accessible for managing the reproduction of small and large ruminants. Artificial insemination (AI) and semen preservation are the main technologies that are used widely. Assessing the fertilization capacity of sperm, sexing sperm, synchronization and fixed-time insemination, superovulation, embryo transfer (ET) and in vitro embryo production (IVEP) are additional techniques that can increase the efficiency of reproductive and pregnancy rates. Molecular DNA markers can also be used for genetic improvement through marker-assisted selection (MAS) as well as to characterize and conserve genetic resources of the animal.

2.1.1. Artificial insemination (AI). Artificial insemination has become the most widely spread biotechnology applied to livestock and especially in cattle production. It remains as one of the most important assisted reproductive technologies [7]. The genetic improvement in a commercial herd depends on the genetic improvement in the AI is about 90%. The utilization of proven sire greatly increases by AI. Artificial insemination is simple, economical, and successful. The successful of AI highly relying on the viability of sperm [3]. Sperm cryopreservation is the technique applied for the sperm to be viable for a longer time. It indicates to the long-term preservation and conservation of biological substance at very low temperatures at -196°C of liquid nitrogen.

2.1.2. Multiple ovulation and embryo transfer (MOET). Embryo transfer biotechnology refers to a step in the process of assisted reproduction in which embryos are placed into the uterus of a female with the intent to establish a pregnancy. The technique generated a lot of interest among the people in the past. This biotechnology enables achieving a greater number of calves from selected females than was possible by applying traditional means of animal production. By increasing the number of calves, MOET has the potential for genetic improvement by increasing the selection intensity on the female. Embryo transfer technique consists of three steps: superovulation by using follicle-stimulating hormones; embryos collection either surgically or without surgically; and transfer of embryos to the recipients. The
advantages of embryo transfer are the preservation of breeds and conservation, disease-free herd creation, economical transport of livestock, for rapid multiplication of the elite female breeding stock, and for research applications.

Implementation of MOET in Indonesia, RC for Biotechnology, Indonesian Institute of Sciences in 1993 began to produce embryo in vivo and embryo transfer in FH cattle (in West Java), PO cattle (in South Kalimantan, Southeast Sulawesi), Simmental cattle (in West Sumatra), Bali cattle (in Bengkulu, West Sumatra, West Nusa Tenggara, Bali, and South Sulawesi). Superovulation treatment with reduced doses gradually of FSH in Hungarian cows results from 2.6 to 5.2 transferable embryos per donor. Superovulation treatment in Brangus cows with a single dose of FSH injection with four treatment doses can produce embryos in the range of 3.3 to 10.7 per donor [8]. Birth of Hungarian dairy cow from Brangus recipient proves that cross embryo transfer can be carried out.

2.1.3. Sperm sexing. By using AI with sexed sperm, hundreds of thousands of calves have been born. Although this technology has been used for many species, the tremendous majority of pregnancies have been in cattle, almost all as a result of sexed sperm and subsequently frozen [9]. AI using sexing sperm was first implemented in West Java, Indonesia in eight different locations: Bogor, Cianjur, Lembang, Sumedang, Garut, Tasikmalaya, Majalengka and DKI Jakarta. AI using sexed sperm in dairy cows results in a sex-match ratio of 81% with S/C 1.37 [10][39], in Simmental cattle has been carried out in West Sumatra resulting in a male sex match ratio of 81.7% with S/C 3.44 [11], and Bali sexed sperm produced in BIBD Puca followed by AI in Enrekang and East Luwu, South Sulawesi resulted in a gender match of 84.09% (sperm X) and 90.98% (sperm Y) with S/C 1.33 and 1.76 respectively [11].

2.1.4. Embryo splitting. Embryo splitting refers to the formation of twins or multiples through the artificial microsurgical splitting of an embryo. After separation, the genetically identical embryos can continue to develop. The morole or blastocyst stages of embryos may be cut into two equal halves by using an inverted microscope connected with a micromanipulator and a microsurgical knife before transfer to a surrogate female. Genetically identical animals can be produced by this method. This process seems to duplicate the natural process of the production of monozygotic twins.

2.1.5. In vitro embryo production. Now bovine embryos can be produced by in vitro fertilization technique. Primary oocytes were collected from antral follicles of ovaries can be induced to lead the maturation process. however, in vitro maturation system must ensure that the resulting oocyte is capable of having normal fertilization and produces a zygote competent of developing to term after embryo transfer. The practical use of in vitro embryo production (IVEP) under field conditions is limited by high production costs and the low overall efficiency. The rates of maturation of 70% to 90%, fertilization of 60% to 70% and cleavage of 40% to 50%, and moderate to low rates of blastocyst formation of 15% to 30% and calf production of 10.5% have been reported [12]. The efficiency of blastocyst production in buffaloes is much poorer than the 30% to 60% reported for cattle [13]. Therefore, IVEP must be improved before it can be widely used in cattle and buffaloes in developing countries.

2.1.6. Embryonic stem cell. Embryonic stem cells (ESCs) are stem cells derived from the undifferentiated inner cell mass of embryo which is harvested from the donor mother animal. Stem cells are pluripotent cells that have the ability to self-replicate and grow into specialized cells. It can be found at different stages of fetal development and are present in a wide range of adult tissues. Stem cells are manipulated in the laboratory in order to make them accept new genes that can then change their behavior. This process includes removing the donor mother’s ovaries and dosing her with progesterone, changing the hormone environment, which causes the embryos to remain free in the uterus. After 4–6 days of this intrauterine culture, the embryos are harvested and grown in vitro culture until the inner cell mass forms egg cylinder-like structures [14].
3. Research and application of biotechnologies in the cattle farm industry in Indonesia

Applying technology to support livestock production is an integral part of viable agriculture in multi-corporation systems. Livestock is part of a weak ecosystem and a rich source of animal biodiversity, as local breeds and species have genes and traits of excellence. Molecular markers are progressively being used to understand and select the particular genes that guide to these desirable traits and it is now potential to select superior germplasm and disseminate it using artificial insemination, embryo transfer, and other assisted reproductive technologies. These technologies have been used in the genetic improvement of livestock, particularly in cattle.

3.1. Genetic study in the Sumba Ongole cattle

Sumba Ongole (SO) is one of the local Indonesian cattle breeds originally Bos indicus species [15]. The existence of the SO cattle in Indonesia began since the Indian Ongole breed was imported from India in 1914 and centralized in Sumba Island (East Nusa Tenggara Province) for breeding programs [16]. The phenotype characteristics of the SO cattle are closely similar with the Ongole Grade’s cattle (known as PO cattle and spread out across in the Java Island), and it is difficult to identify these two Ongole breeds based on the phenotypic parameters because the PO cattle is crossbred of uncontrolled mating of the SO cattle breed and Java cattle breed [17] or other Indonesian local breeds [18]. In order to resolve the difficulties in the SO and PO identification, it has been reported that the TGLA227 and ETH225 loci (based on microsatellite markers) and several haplotypes based on the mitochondrial DNA can be considered as the differentiation factors between the SO and PO cattle breeds [19].

The SO cattle have excellent potential to gain higher dressing percentage (>50%) compared with other local cattle breeds in Indonesia [20]. However, this quality can be improved by selective breeding. There are two methods in order to generate the SO cattle population with the best productivity i.e. conventional and genetic selection. The conventional method in the SO cattle can be done by evaluating body weight and size [21]. According to Putra et al. [22], weight is the most important trait in the selection of beef cattle. Meanwhile, genetic selection is conducted through the identification of genetic markers that encode specific economical traits, along with its polymorphism in the population [23]. To this account, the gene that has been extensively studied in the SO cattle is the growth hormone (GH) gene [22-24]. The expression of GH in different individuals of SO in one population may differ, due to mutation and environmental causes. This phenomenon is called polymorphism. In an attempt to detect the occurrence of polymorphism and to select cattle with promising GH in such a population, a molecular approach was conducted. One of the approaches is marker-assisted selection (MAS). This method is a combination of phenotype and genetic marker data.

One of methods to detect polymorphism in the SO cattle GH gene is the PCR-RFLP method as reported [24] and [20]. Both studies prove the occurrence of polymorphism in the SO cattle GH gene. There are 28 SNPs identified within the GH gene i.e. 21 SNPs were found to occur in the intron region, 4 SNPs in the exon region, and 3 SNPs in the 3’ end region [25]. There were 17 SNPs in the SO cattle GH gene that were caused by a novel mutation. In addition, the novel SNP g.1395insC was found only in the SO cattle. The SNP g.1642C>G causes an amino acid change from leucine (Leu) to valine (Val). However, the polymorphism of the GH gene was not associated with several growth traits in the SO cattle [23]. Therefore, information about other genetic marker candidates needs to be explored. The other genetic marker candidate that has been studied in the SO cattle was Leptin (LEP) gene. Leptin is a protein involved in the regulation of fat metabolism, feed intake, hematopoiesis and whole body energy balance in cattle [26]. A total of 17 SNPs in the 3’ flanking region (3506 - 4019 bp) of the SO cattle LEP gene were identified [22] and five SNP’s (g.C3558T; g.G3566T; g.A3567C; g.G3574A; g.C3575A) were common mutation in the SO cattle LEP gene.

An estimation of the most probable producing ability (MPPA) value based on the calves performance in the SO cattle was reported [27]. High of r value (r > 0.30) was obtained at the body length (BL). Chest girth (CG), withers high (WH) and birth weight (BW) had a moderate value (0.10 < r < 0.30) of repeatability. Based on the highest birth records, the MPPA value of the cumulative calf birth’s performance of cows was 4.64 (cow number 3770) and the lowest was -4.64 (cow number 2283). The
BW of birth had a moderate value of r (0.10) and could be used as selection criteria accurately in the SO cattle population.

A performance test in the SO cattle population was also has been conducted using 25 males and 25 females of Sumba Ongole (SO) cattle as reported [28]. The performance test was conducted in three periods (2014, 2015 and 2016) for about 535 days. Cattle with age between 300 to 600 days old were used for evaluation, showed that the heritability value of yearling weight (YW365) was 0.77±0.68 as a high category. The highest breeding value of YW365 was 41.89 kg (female) and 66.05 kg (male). The average of corrected final weight (CFW) weight were 159.80+37.73 kg (female) and172.55+34.22 kg (male). The performance test in this study obtained six A class (1 bull and 5 cows) based on the standard minimum of body measurements for SO cattle [28]. Non-genetic parameters in the SO cattle such as weight [22], body size [21,22], calf birth and also calf performance can be used as the selection parameter in the SO cattle population. Bodyweight is the first and most important information regarding the calf potency in becoming healthy and beneficial cattle. Based on Putra and Agung [21] report, body size parameters such as withers height (WH), chest girth (CG), and body length (BL) can be used to evaluate the SO cattle population. Body length-based selection is more accurate than withers height and chest girth.

3.2. The genetic study in Pasundan cattle
Pasundan cattle is one of Indonesian native cattle that adapted well in West Java province. These cattle were decided as one of Indonesian native cattle since the year 2014 through the decision of Indonesian Ministry of Agriculture No: 1051/Kpts/SR.120/10/2014. According to the twelve microsatellite locus, Pasundan cattle had similarities with Madura cattle (Bos indicus) [15]. Despite, according to Cytochrome b (mtDNA) sequences, the Pasundan cattle was had similarities with Bali cattle (Bos javanicus) [29]. In addition, Pasundan cattle had three types of protein albumin [30]. However, most of the Pasundan cattle had a phenotypic characteristic that similar to Bos indicus (humped) and Bos javanicus (reddish coat color with a whitish color in rump pack and legs) [31].

Previous studies reported that the Single Nucleotide Polymorphism (SNP) was not found in many candidate genes for productive traits of Pasundan cattle in the exon 1 of Insulin-like Growth Factor 1 (IGF1) gene and promoter region of Adiponectin (ADIPOQ) gene and 5'UTR of Thyroglobulin (TG) gene [22,32,33]. Meanwhile, SNP in the many candidate gene of Pasundan cattle were showed in 5'UTR of endothelial differentiation sphingolipid G-protein-coupled receptor 1 (EDG1) gene, exon 3 of Calpastatin (CAPN) gene, exon 3 of Fat Mass and Obesity Associated (FTO) gene, intron 2 to exon 3 of Leptin (LEP) gene, exon 6 of Pituitary specific transcription factor 1 (Pit1) gene and exon 10 of Growth Hormone Receptor (GHR) gene [32-37]. However, the genetic diversity in FTO, LEP and Pit1 genes of Pasundan cattle were low and not effective as the molecular selection.

3.3. First crossing of Belgian Blue cattle in Indonesia
The difficulty of increasing population and productivity were the big problems faced in the Indonesian beef cattle industry. In order to increase productivity, the crossing program between European cattle breed and local cattle breed is an option that can be applied in Indonesia. Research Center for Biotechnology, Indonesian Institute of Sciences has collaboration with PT. Karya Anugerah Rumpin (PT. KAR) Farm to initiate a beef cattle breeding program based on an industrial approach since 2012. It has been reported that a number of semen of the Belgian Blue cattle were introduced into Indonesia and used in the crossing program in 2013 [38]. Furthermore, the deletion of 11 bases in the third exon of the myostatin gene in the Belgian Blue cattle semen has been confirmed [38].

The first F1 generation of the Belgian Blue in Indonesia in KAR Farm, thus allowing analysis of the inheritance of the genetic marker for the “double muscling” phenomenon. The results in this study confirm that the F1 generation of the Belgian Blue does indeed hold the heterozygous myostatin gene [39]. At present, the Balai Embrio Ternak (BET) Cipelang has also developed BB cattle where the embryos are imported directly from Belgium. The first time in Indonesia a BB male calf produced by embryo transfer (ET) at BET Cipelang was given the name "Gatot Kaca" (ear tag no. 881707). The calf's
birth weight reached 62.5 kg and came from a BB male named "Adajio De Bray" (No. ear tag: BE 255530745) [40].

4. Major constraints on applying the technology in Indonesia

The application of new molecular biotechnologies and new breeding strategies to the livestock breeds used in smallholder production systems in Indonesia is constrained by a number of factors. The major constraints on applying the biotechnology in animal production in Indonesia which include:

- Lack of complete and an accurate database on livestock
- Biodiversity within species and breeds
- The models of biotechnological intervention differ between advanced farmers and expanding farmers.
- The uniqueness of animal breeds, each has its own distinct developmental, nutrient utilization characteristics, production, and disease resistance.
- The absence of mechanism between institutions, industry, and universities, which is important to translate technologies into products
- The high cost of technological input
- Lack of clear policy and commitment from the government

5. Conclusions

Biotechnology in animal production in Indonesia has been applied only in a few areas due to reasons related to economic growth. Applying biotechnology has benefitted in the animal improvement and economic returns to the livestock entrepreneurs, however, Indonesia as one of developing countries has to address issues relating to infrastructure, funding, political commitment, and trained manpower.

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