Muhammad Abubakar · Ali Saeed
Oguz Kul *Editors*

The Role of Biotechnology in Improvement of Livestock

Animal Health and Biotechnology

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ISBN 978-3-662-46788-6 ISBN 978-3-662-46789-3 (eBook)
DOI 10.1007/978-3-662-46789-3

Library of Congress Control Number: 2015935736

Springer Heidelberg New York Dordrecht London

Printed on acid-free paper

Springer-Verlag GmbH Berlin Heidelberg is part of Springer Science+Business Media (www.springer.com)

Preface

Biotechnology is undoubtledly one of the most important scientific areas in the future of mankind. In the second millennium, the cooperations between the scientific branches that are much more open to novelty such as molecular genetics, genetic engineering, proteomics, and targeted production technologies, have generated great opportunities for human and animal welfare, nutrition and health issues. Novel biotechnological products and techniques developed for the improvement of existing classical agricultural and animal husbandry will also be environment and nature friendly.

As biotechnology is a complexity of all the areas of cell and tissue culture, molecular biology, microbiology, genetic sciences, and almost all engineering technologies, it is targeted at development of new technologies and/or products that are closely related to productivity of animals, animal yields, or health. For example, recombinant DNA technologies have been commonly used to produce specific enzymes and proteins that are not sufficiently synthesized by the organisms themselves or that are not present in nature. Whereas the use of biotechnology in veterinary practice finds a very wide range from animal health to animal husbandry, generally it takes enormous and important duties for improvement of quality and quantity of animal products, development of industrially integrated biological products, reduction of waste products of animals, and environmental sensitive solutions. In this book, it is also emphasized the positive contributions of veterinary biotechnology on the following subjects:

- Embryo cloning, cryopreservation, embryo sexing, and transfer techniques
- Animal health, DNA, and recombinant vaccine technologies
- Recombinant drug, enzyme, and protein production using transgenic techniques
- Biotechnological approaches to animal nutrition and feed efficiency
- Impacts of biotechnology on the environment

Biotechnology has taken the first place among all the sciences that hit the mark in the twenty-first century, having close collaborations with nearly all other biological, engineering areas; it is an interdisciplinary branch. Thus, I and all contributing

authors believe that this book will be of use to students and experts who are studying veterinary, animal husbandry, biology, chemistry, medicine, pharmacy, agriculture, and other disciplines in engineering.

Contents

Chapter 1
Biotechnology and Animal Reproduction

Ahmad Yar Qamar, Aman Ullah Khan, Aatka Jamil and Muhammad Abubakar

Biotechnology has great impact on breed improvement, reproductive rate, and animal production.

The most common reproductive applications that are integrated with biotechnology are artificial insemination (AI), semen preservation, fertilization capacity of sperms, sperm sexing, synchronization and fixed-time insemination, superovulation, embryo transfer (ET), and in vitro embryo production (IVEP).

1.1 Artificial Insemination

Artificial insemination has been practiced on many domestic animals for hundreds of years. It is one of the earliest reproductive biotechnologies and permits the use of superior males for breeding purposes. This technique involves semen collection from superior males, its dilution, freezing, and deposition in the female reproductive tract. The first successful artificial insemination (AI) was reported in a water spaniel bitch in 1780 by the Italian scientist, Spallanzani and got three puppies. Spallanzani's work was confirmed 2 years later by another scientist Rossi (Roberts 1971). After initial work on bitches, AI was done in mares by Pearson. The AI technique in different farm animals is based on AI techniques of horses developed by Ivanow (1907).

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M. Abubakar et al. (eds.), *The Role of Biotechnology in Improvement of Livestock*,
DOI 10.1007/978-3-662-46789-3_1

1.1.1 Semen Collection Methods

There are many methods of semen collection in domestic animals. Primitive methods involved semen collection directly from the vagina of an estrum female with the help of a spoon or syringe with a long nozzle. But the semen was contaminated, contained mucus, and the quantity recovered was minute. It had also a limitation of estrus female. Rowson in 1947 devised a method of making fistula in the urethral opening leading toward the vaginal fornix. Rowson's method involved semen aspiration directly from vaginal fornix by using catheter after natural breeding but it resulted in fibrosis, urinary tract infection, and also had the same limitation as the primitive method. After that, massage method and electro-ejaculator methods were developed.

1.1.1.1 Massage Method

This method was devised in the bull for the first time by Case in 1925 and modified by Miller, Evans, and Goodwin in 1934 (Roberts 1971). This method involves the massage of ampulla and seminal vesicle per rectum.

Indications

- Used in the case of aged bulls unable to mount.
- Used for crippled bulls.
- Used for bulls with decreased libido or with the problem of impotency.
- Used for bulls that are unwilling or unable to copulate.

Procedure

- Restrain the bull properly, handle it quietly, and keep it relaxed.
- Wash, rinse, and dry with a brush and cotton pledgets or with a clean towel the prepuce and the preputial hairs and the region around the preputial opening with warm physiological saline.
- During washing, stroking of sheath should be done to induce urination, which will not cause any contamination during collection.
- Operator, wearing a glove gently inserts a lubricated hand and forearm into the bull's rectum emptying it of feces.
- Massage the vesicular gland a few times in backward and downward fashion toward urethra. This will result in release of cloudy fluid containing few spermatozoa.
- The ampulla is then massaged in the same fashion. The semen is stripped with pressure against floor of pelvis.
- Sometimes pelvic urethra is also massaged.
- Another person holds a rubber cone attached to a collection vial placed in a plastic bag attached to a metallic ring about 7.5 cm in diameter with a long handle.

Limitations of Massage Method

- Skill and experience is needed for massage.
- Semen samples collected are not usually clean and contain many bacteria, as semen dribbles through the prepuce and drips from preputial hairs.
- More secretions of accessory sex glands and low sperm concentration.
- Sometimes sample may also be contaminated with urine.

1.1.1.2 Electro Ejaculator Method

This technique was first described and used in rams by Gunn in 1936. The probes used for this purpose are of different sizes and shapes depending on the species. This technique is painful for bulls and so was criticized by the Animal welfare personnel (Roberts 1971). That is why, it is only used in animals as in the below indications.

Indications

- Used in the case of aged bulls unable to mount.
- Used for crippled bulls.
- Used for bulls with decreased libido or with the problem of impotency.
- Used for bulls that are unwilling or unable to copulate.

Procedure

- Restrain the bull properly, handle it quietly, and keep it relaxed.
- Wash, rinse, and dry with a brush and cotton pledgets or with a clean towel the prepuce and the preputial hairs and the region around the preputial opening with warm physiological saline.
- The preputial hairs should be clipped.
- Operator, wearing a glove, gently inserts a lubricated hand and forearm into the bull's rectum emptying it of feces.
- Now the probe is inserted into the rectum placing it in the midline against the floor of rectum. Probe should also be lubricated with a noninsulating material like "K.Y" jelly.
- After the proper placement of probe, 3–5 V of current is applied for 3–5 s. It will result in erection and dripping of seminal fluids.
- After 3–5 s of current application, animal is given rest for 3–5 s.
- After resting, again the same amount of current is applied for the same time, then again rest is given. This process is repeated at least 5 times.
- Now current is increased up to 10–15 V. This current is applied for 3–5 s and after that the animal is given rest for 3–5 s as done with the low voltage. This will result in semen ejaculation.
- High voltage of 10–15 V is applied for 5 times with intervals as applied in low voltage.

- Another person holds a rubber cone attached to a collection vial placed in a plastic bag attached to a metallic ring about 7.5 cm in diameter with a long handle.

Limitations of Electro Ejaculator Method

- Skill and experience is needed for massage.
- Semen samples collected are not usually clean and contain many bacteria, as semen dribbles through the prepuce and drips from preputial hairs.
- More secretions of accessory sex glands and low sperm concentration.
- All bulls stiffen and show arching of back due to pain. Sometimes, bull may lean to one side or raise and extend one or both the rear limbs.
- This may lead to ataxia.
- Sometimes, choking may lead to death of animal due to pressure exerted by rear limb extension.

1.1.1.3 Artificial Vagina Method

This is the most widely used method. The early models made by Russians consisted of a bag like artificial vagina placed inside the vagina of a cow or the dummy. The AV used these days were developed in England, Perry, and Maule (Stephen). It is preferred over the other methods because the semen collected by this method is clean; complete ejaculate is obtained which is closer to natural ejaculation. There are two types of artificial vagina for bulls: a triple layer type or winter-type AV and a double layer type.

The artificial vagina of a *triple layer type* consists of three major layers, an outer casing made of plastic, a rectangular inner sleeve A made of rubber and a triangular inner sleeve B made of rubber. The inner sleeve A is fitted to the outer casing, and the inner sleeve B is fitted to the inner sleeve A. At one end of the inner sleeve B, there is a collection vial. Semen flows into the collection vial. This arrangement protects semen from temperature shock due to changing of temperature. But the main disadvantage of the triple layer AV is that it is longer and heavier compared to the double layer type.

The artificial vagina of a double layer type is shorter and lighter than the triple layer AV. It is used in tropical and warm regions. Due to shorter size it is easier to use. It consists of an outer casing, inner rubber liner, a cone, a collection vial, and an insulating jacket. About half to two-third of the chamber formed between inner rubber liner and the hard casing is filled with warm water. The water should be 125–180 °F, 50–70 °C. At the time of collection, the temperature of AV should be between 40 and 50 °C (MacMillan et al. 1966). Only small amounts of lubricant should be used for lubricating the inner liner. More quantity of lubricant will contaminate the semen. For this purpose, white sterilized Vaseline, K.Y. jelly, or pure white mineral oil are used (Fig. 1.1).

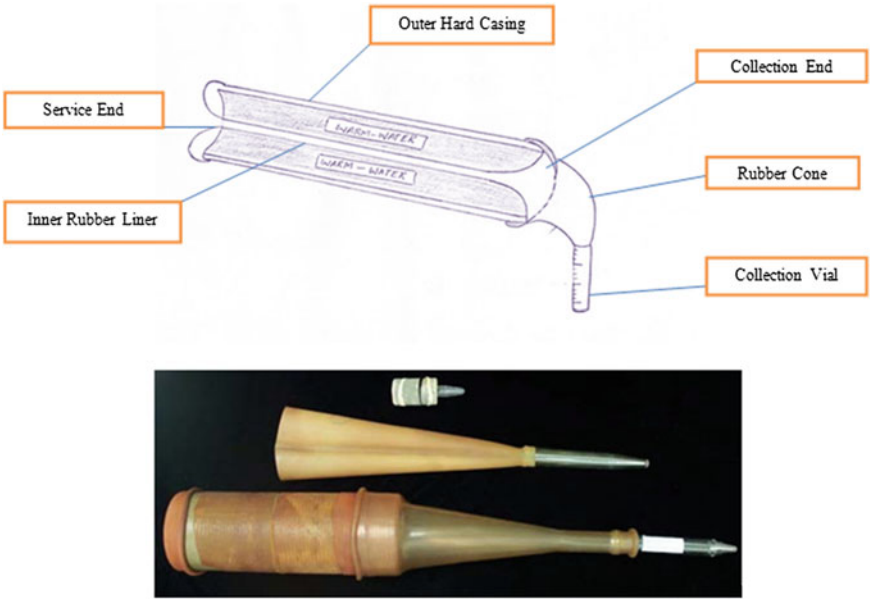

Fig. 1.1 Double layered artificial vagina

1.1.2 Advantages of Artificial Insemination

1. Artificial insemination not only increases the use of superior male animals but also makes their use more efficient. More people can be benefited from superior male. Use of the proven sires in dairy herds markedly increases milk production up to 30 % compared to natural breeding, Van Vleck.
2. Artificial insemination helps in great genetic improvement of farm animals. The selection and efficient use of superior bulls improves production.
3. Artificial insemination helps in controlling different venereal and other diseases like trichomoniasis, Vibriosis, brucellosis, etc.
4. The danger and expenses of keeping and handling bulls that prove to be inferior males can be eliminated.
5. It is easier to transport semen doses over long distances than to transport male animals.
6. Artificial insemination makes it possible to use the semen even after the death of a male.
7. Widespread use of artificial insemination in the dairy industry helps in proper breeding records.
8. Artificial insemination makes possible breeding of animals with size differences without injuries.

9. Artificial insemination made the use of those sires that are not capable of copulating, like aged or crippled sires.
10. It is a pre-requisite for embryo transfer.

1.1.3 Disadvantages of Artificial Insemination

1. Artificial insemination is an advanced and sophisticated technique, so well-trained personnel are required to supervise semen collection, examination, extension, freezing, shipping, and insemination of females.
2. Widespread use of artificial insemination increases the possibility of transmission of genetic abnormalities, for example COD, spastic syndrome, poor conformation especially of feet and limbs, and lack of libido.
3. Artificial insemination uses a limited number of elite bulls. This limited gene pool may improve milk production but it has a reverse effect due to increased inbreeding, which results in genetic abnormalities because of expression of recessive genes.

1.2 Sex Sorted Semen

Sex of the fetus is determined by the sperm because the sperm may carry either X or Y sex chromosome. Sperm having X sex chromosome when fertilizes an oocyte will result in a female and a sperm having Y sex chromosome when fertilizes an oocytes will result in a male offspring. The desire to separate X and Y bearing sperms is driven by the fact that one sex has more economic importance than the other for certain species. As in the dairy industry, the female calves are more important than the males because of maximum utilization of AI. As the major income of a dairy farm comes from milk, so it is advantageous to have more female calves that will become future producers (Senger 1999).

1.2.1 Procedure

The technique used for separation of X-bearing sperms from Y-bearing sperm is known as "Flow cytometry or cell sorting". Experiments have resulted in 80–90 % of successful separations of sperms in rabbit, cattle, and swine. It is well known that the X and Y chromosomes have different quantities of DNA. It is said that the X-bearing sperm has 2.8–4.2 % more DNA compared to Y-bearing chromosome depending on the species (Senger 1999). On the basis of difference of DNA, we can separate the X-bearing sperms from Y-bearing sperms. For this purpose, a DNA

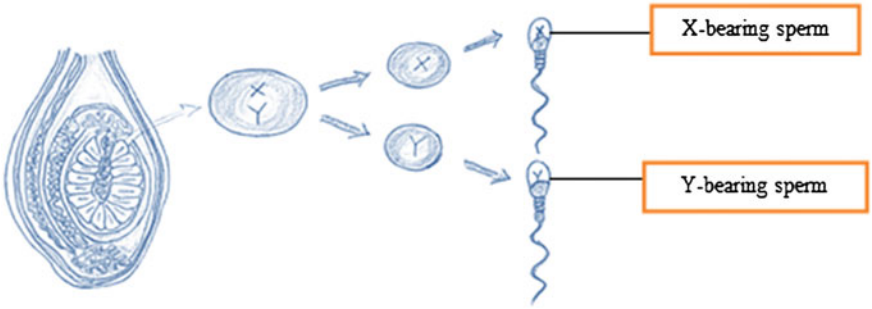

Fig. 1.2 X and Y bearing sperms produced by testes

stain or dye is used called Fluorochrome. The X-bearing sperm will take more DNA dye compared to the Y-bearing sperm. Vital stains used for sperm staining have a property of emission of light of specific wavelength when excited or activated by a light of specific wavelength.

Step 1: Collection of the semen from the male by using different methods as explained earlier (Fig. 1.2).

Step 2: Treatment of semen with Fluorochrome (Sperm staining dye). X-bearing sperms will take on more stain compared to Y-bearing sperms (Fig. 1.3).

Step 3: Once the spermatozoa enter the flow cytometer chamber, they pass single file through a small nozzle. After staining, the stained spermatozoa are excited by a laser beam. As a result of excitation, the X-bearing sperms emit more light compared to Y-bearing sperms. Sperms will emit light of different wavelength depending on their liveability and DNA contents. Dead sperms emit a very low beam of light when excited by laser beam, so they are easily differentiated (Fig. 1.4).

Step 4: After being excited, sperms pass through a light sensing device that is coupled with a computer. This device will determine the amount of light emitted by sperms and also order the passage of each sperm through a column below the nozzle.

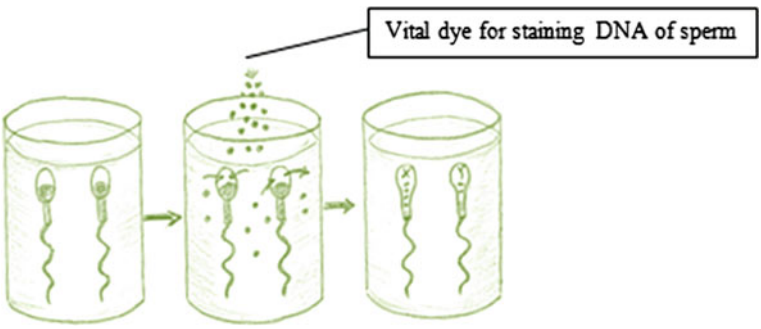

Fig. 1.3 Ejaculated sperm treatment by DNA dye Flurochrome