

POTENTIALS OF EMBRYO TRANSFER AND THE RELATED TECHNOLOGIES IN THE FUTURE OF ANIMAL BREEDING AND PRODUCTION IN OUR COUNTRY (TANZANIA)

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SUMMARY

Over the world, a great deal of research is being conducted with an objective of intensifying the productive efficiency of animals, as a prelude to increasing the availability of food. One of the techniques which is certainly ready to be applied in developing countries like ours is the embryo transfer, which has a significant impact on genetic improvement and facilities for a speedy international exchange of a full genetic material. Other relevant aspect is the need for correct control of embryo transfer, as the base in understanding more advanced technologies such as cloning, embryo sexing and splitting, production of chimeric and transgenic animals, and laboratory production of embryos by in vitro matured, fertilized and cultured embryos. This paper summarizes some basin aspects of the current application of embryo transfer and the related technologies that are capable of intensifying animal reproductive capacity and in thus their possibilities of adoption in a developing country like Tanzania.

INTRODUCTION

The World Bank's standard projection yields world population of 8.3 billion in the year 2025. Almost 7 billion people (85% of the planet's population) will live in the 33%, respectively, in 1950 (Cassen, 1994). Accommodating growing numbers of people against a fixed endowment of resources has obliged human beings to intensify agricultural production to the maximum, and it is a big challenge lying ahead in many developing countries. Every existing possibility of improving food production outputs must therefore be rationally exploited.

In order to achieve this goal, new biotechnologies aimed at increasing the reproductive efficiency of animals have, and still are, being introduced, with highly genetic improvement and facilities for international exchange of genetic material avoiding transmission of diseases, offers a

very valuable mechanism. Not less important are the basic research advances in molecular biology and reproductive physiology (such as immunological techniques for the determination of sexual hormones), all of which have created the possibility and reality of gene mapping and characterization, production of "clonal" lines of animals, *in vitro* maturation (IVM) and fertilization (IVF) of embryos, just to mention a few. These advances portrays an evidence of having a major impact upon future strategies for the production of food from animals that we ought to discuss some of them at this time.

Therefore, this paper attempts to highlight the current state of embryo transfer and the related technologies which are meat to intensify the reproductive efficiency of female bovines, from an economic and productive points of view.

SELECTION OF DONORS AND RECIPIENTS

The value of a donor cow (a female from which embryos are obtained) can be defined according to different standards, although without the influence of market trends, cattle intrinsically are worth only the meat, milk and by-products they produce. In the past especially, and currently to some extent, scarcity and promotion have had more influence on price than has genetic value. However, true genetic value—the ability to transmit desirable traits in cattle—is the most important long-range consideration in ET technology.

Donor selection should be based on these criteria: i) genetic superiority, ii) reproductive ability, and iii) market value of the progeny (Elsden and Seidel, Jr., 1985). When selecting genetically superior beef cow as a donor, such objective traits as maternal breeding value, weaning breeding value and yearling breeding value need to be considered. In dairy cattle, a donor with high cow index (like milk production, maternal behaviour and lactation period) should be selected. It should however be noted that the best producers are not always good reproducers, particularly due to the endocrine and metabolic disorders associated with high production capacities. Such a tendency has been reported by various researchers (Lerner *et al.*, 1986; Caral *et al.*, 1986; de Armas, 1990), who have described a whole series of factors adversely affecting the superovulation response (breed, age, post-partum reproductive status, individual sensitivity to gonadotrophins, management, etc) which are inherent to the animal. Consequently, in selecting an effective donor, such factors need to be rationally accounted for.

For recipients, unlike the donors, do

not have to be animals of high genetic value, since their role consists of only operating as biological incubators. Cows and heifers could be indiscriminately used as recipients. Despite this, results are showing that heifers are the category to choose on account of their better fertility (de Armas *et al.*, 1986). though their physical state, age and oestrus cyclicity are of paramount importance in attaining the desired objective.

Both donor and recipient cows need to have correct handling, feeding, and health control, in order to ensure that they are in the best possible reproductive state.

SUPEROVULATION

By definition, superovulation is the term used for stimulation of a larger than normal complement of follicles to mature into large follicles ready for ovulation (or simply, the induction of multiple ovulation through the use of gonadotrophins). It is one of the most important problems in the practice of ET since the availability of embryos suitable for transfer depends on its success.

For many years, the standard drug for superovulation was pregnant mare's serum gonadotrophin (PMSG), and it still is in the most parts of the world due to its low cost, widespread availability and easy application. However, treatment with follicle stimulating hormone (FSH) have also been introduced with somehow superior results regarding the corpora lutea (CL) and viable embryos than does the PMSG (Varisanga, *et al.*, 1993). One of the main problems with FSH is the number of injections to be administered, as a result of its short half-life span in the body. Normally it is subscribed in two injection per day for about 4-5 days in order to maintain sufficient levels in the blood to act on the ovarian follicles.

The treatment described by Philipo and Rowson (1975), using a combination of gonadotrophin and prostaglandin or its synthetic analogues (PGF_{2α}) applied in the mid-luteal phase, is still frequently used. Independent to the hormone chose, both PMSG or FSH should be injected intramuscularly (i.m.) within the luteal phase (Day 8-14 of the oestrus cycle; Day 1 of oestrus = 0). Many authors have found no difference in results, but all agree on applying the superovulation treatment on days 10 to 12 of the cycle (Thomas and Williams, 1996).

The optimum dose of PMSG is considered to be 2,500-3,000 IU (heifer) and 3,500-4,000 IU for cows. This treatment is followed by two doses of PGF_{2α} (25-30mg) at 48 and 60 h from its beginning, though some prefer to admit just a single dose. FSH is usually given in a decreasing dose twice a day for 4-5 days; for instance, 5-5, 4-4, 3-3 and 2-2 mg im. (for a 4 days scheme), to give a total dose of the hormone in the range between 30-40 mg. Each animal is to be given PGF_{2α} (25-30mg) shots 48 h after the initial gonadotrophin (FSH) injection, at the rate of 2 to 3 injections (i.m.) per day.

With regard to the superovulation treatments, the variability in response to a standard superovulatory treatment is both wide and notorious. As pointed out by Hasler (1992), the outcome of superovulation treatment has shown little improvement in the past 10 to 15 years. Although researchers differ in their estimates, there is ample evidence in many instances to show that no more than 5-7 embryos of transferrable quality are recovered per flushed donor. Various studies have been carried-out with an progestagens, hCG (human Chorionic Gonadotrophin), gonadotrophin releasing hormone (GnRH0, and the anti-PMSG sera; and many are still

being conducted to improve the efficiency of superovulation.

FERTILIZATION OF THE OVA

Both natural mating or artificial insemination (AI) can be employed to fertilize the released ova after superovulation. Among others, the main concerns in this aspect includes the establishment of the right time to carry out the AI or natural service to the donor, number of services required and the quality of semen. Nevertheless, in many ET programmes, usually 2 or 3 services (one or two doses) are given, at 12, 24 and 36h after the start of oestrus (price, 1995). All in all, donors must show a standing estrous at the time of AI, and the semen employed must be of both high quality and in a way, more concentrated.

EMBRYO RECOVERY (COLLECTION)

Bovine embryos do not arrive in the uterus until the 5th day after oestrus, so the choice of the technique to be used (surgical or non-surgical method), depends on the moment and purpose of such embryos collection. For a routine ET, embryo collection is currently performed by the non-surgical method between days 6 and 8 after oestrus, since at that point in development it is possible to freeze or carry-out other micromanipulations of the embryos with good gestation results post-transfer. Moreover, the non-surgical method of collection is preferred because of its reliability, easy to conduct and low cost, compared to the surgical method.

For the non-surgical method, two basic items of equipment are routinely used; the first consists of a rubber catheter (Folley) which has a balloon to block the

uterine lumen so that the flushing medium insufflated does not escape and thus be recovered solely through the catheter. The second consists of a metal tube with a balloon at the end for the same purpose, and through this, a flexible catheter can be introduced up to the tip of the uterine horn. In essence, this is a three-ways catheter; the first channel is for the air-balloon, the second for injecting flushing medium and the third for recovering it (Holy, 1987).

Each born is flushed with about 500 ml of the flushing medium, which is made of phosphate-buffered saline (PBS) solution supplemented with 2-5% of foetal calf serum (FCS). When irrigation is completed, all the collected medium except 100 ml in the bottom of the collecting bottle is drained off gradually using silicon siphon, after the collect have allowed to settle down for approximately 30 minutes. The remaining flush medium is transferred to 3-4 large Petri-dishes for examination.

EMBRYO EVALUATION

After collection, the next step is the search and evaluation of embryos, which is done under a stereomicroscope. Obviously, the same collection often includes embryos at different stages and of different qualities; unfertilized ova, retarded embryos, some with defects on the zona pellucida, degenerated and viable ones.

Although there are many techniques for evaluating embryo's viability, most of them are complex and require both time and sophisticated equipments, which certainly jeopardizes the future development *in vivo* of these embryos. The morphological criterion is therefore of primary importance in assessing the suitability of the embryos for their posterior transfer. Equally important is the knowledge of the

chronological development of embryos, including the normal characteristics of its developmental stages, so that defects that determine its classification as transferrable or not could be readily distinguished (Salano, 1988).

Generally, when assessing the embryo, one needs to consider such elements as: sphericity, general conformation, opacity, visible structures, state of cellular aggregation, variation in size of cells, detachment of the cells, integrity of the blastomeres and the stage of development. Bearing in mind these elements, recovered embryos can be classified according the their morphological aspects as follows:

- A **excellent**, and ideal embryo which has a perfect morphological development at each stage.
- A' **good**, with slight imperfection such as few extruded blastomeres, irregular shape and few vesicles.
- B **fair**, presence of extruded blastomeres, vesicles and few degenerated cells are found.
- C **poor**, severe problems such as numerous extruded blastomeres, degenerated cells are of different size and large number of vesicles; though the embryo appears viable.
- D **non-transferrable embryo**, are the ones which are either totally degenerated cells, too young cells or non-fertilized ova.

One more point to note is that embryo quality categorization is very subjective and qualitative. One needs to be

able to differentiate which embryo is better than the other among the whole recovered embryos.

Once located and classified, the embryo should be maintained in PBS + 20% FCS until transfer, freezing or micromanipulation. It has been observed that the time passed in culture medium is inversely proportional to the results of gestation after transfer, which means that a short period of time between collection and transfer ensures best fertility rates.

EMBRYO TRANSFER

Similar to collection, non-surgical transfer has superseded surgical method of embryo transfer, and at present the results are comparable. There are three basic items of equipment for this: i) the Cassou gun, ii) the Hannover embryonization model and iii) the concentric tubing equipment. All these instruments allow the embryo to be introduced through the cervix and then deposited in the uterine horn. Similar gestation rates (between 50 - 70%) can be achieved with either one of these equipments.

The fundamental prerequisites to achieve best results includes; deposition of the embryo at the two-third length of the uterus horn ipsilateral to the CL, fast but careful manipulation of the genitals, the synchrony of the donor and recipients, as well as a strict hygiene. In ET, precise oestrus synchronization between donor and recipient is highly important, because the survival of the embryo depends on a proper synchrony between its own stage of development and conditions in the uterus of the recipient.

Different types of hormones are used to control the cyclicity of the female cattle, but among others, prostaglandin or its

analogues are the most commonly employed in the synchronization of recipients. The other hormones include progestagens administered either orally or in the form of implants. Whether the recipient is naturally on heat or artificially produced, a synchrony should not exceed the range of ± 1 day for a maximum success.

As far as the whole technique for embryo transfer is concerned, Gordon (1994) reiterates that the success and economics of a commercial ET programme greatly depends on the followings:

- i. skill and experience of the ET operator;
- ii. selection and management of recipient animals, which must be cyclic, health and reproductively normal;
- iii. synchrony of estrous between donor and recipient;
- iv. quality of embryos transferred; and
- v. methods employed in embryo handling and transfer on the farm.

FREEZING AND THAWING OF EMBRYOS

On average, 5 - 8 recipients are prepared for one superovulated donor. However, sometimes as many as 15 to 20 embryos are not rare at one collection, making it necessary to establish some effective means for embryo preservation. Recovered embryos can be stored by freezing and thawing them for use when desired. Until recently, pregnancy rates following non-surgical transfer of frozen-thawed bovine embryos have been between 35 and 60% Vitale *et al.*, 1994).

Advantages in freezing embryos include: i) embryos can be stored indefinitely; ii) embryos can be collected

from donors all the years and transferred during the breeding seasons of beef hers; iii) surplus embryos can be stored as recipients become available; iv) breeds of cattle on verge of extinction can be preserved; and v) half embryos can be frozen, and the other half transferred. However, the disadvantages include: i) expense of a freezing equipment and trained technician; ii) only embryos of high quality are suitable for freezing, and thus about 15% of otherwise transferrable embryos must be discarded or transferred without freezing; and iii) about one-tenth of the frozen-thawed embryos are severely damaged (Elsden and Seidel, Jr., 1985). Therefore, the reasons for storage of embryos by cryopreservation must be sufficient to overcome these losses, because the technique offers an economic alternative only under special circumstances.

Research in freezing techniques has included numerous studies dealing with the type and concentration of cryoprotectant, cooling and freezing rates, seeding and plunging temperatures, thawing temperatures and rates and techniques used to ensure cryoprotectant removal. The most commonly used freezing method involves placing the bovine embryo, at the blastocyst stage (7 days old embryo), in a concentrated glycerol solution [1.4M in PBS supplemented with FCS or bovine serum albumin (BSA)] at room temperature for a 20 min equilibration period. Bovine serum is generally added to PBS at about the 15% level and BSA at 4 mg/ml. The straws are usually cooled abruptly from room temperature to 0°C and seeded at -4°C. Seeding is the term used to describe the controlled initiation of the ice formation at slightly supercooled temperatures, generally by touching the wall of the straw with very cool forceps. After seeding, the ice forms quickly throughout the entire straw, and the cooling is

continued at rate of 0.3°C/min to -35°C, when the embryo is plunged into liquid nitrogen (-196°C) for storage.

With respect to the addition of the cryoprotectant, some researchers recommend passing the embryos through successive steps up to the concentration of 0.1M, while other advocates the use of a direct passage to this concentration.

Thawing is relatively easy, and it consists of putting the straw into a recipient with water at 37°C (water bath), followed by the procedures to remove the cryoprotectant. This is carried-out by passing the embryo through increasing concentrations of the cryoprotectant or by a sucrose solution at 0.25M to be finally placed in PBS + 20% FCS or BSA and evaluated before transfer.

ADVANTAGES OF EMBRYO TRANSFER

The first commercial advantage of cattle ET consists on facilitating genetic improvement in the cattle industry by obtaining a larger number of calves from animals of high genetic quality. By using ET, one superior female animal can be made to produce up to 40 superior progenies per year, compared to only one per year from natural mating method or AI. That way, the very few breeding centers in our country (like Mpwapwa, Mlingano, and Tengeru) could be appreciably more efficient if instead of rendering AI services or whole sale of heifers, they would engage on supplying embryos to our poor and needy farmers.

The technique of freezing embryos is more than convenient, for it enables them to be moved from one country to another (or even within the country) while in their frozen state, thereby reducing the need for

long distance cattle movements, with their attendant welfare problems. In other words, ET offers access to the highest-quality genetics at a fraction of the cost of importing a live animal. More than that, animals introduced as embryos are likely to adapt more readily to their new environment than those introduced as grown animals; at birth, calves can receive passive immunity to local diseases from their surrogate mothers (native zebu cattle) and their immune system will develop in the appropriate environment. Such observations have been reported in Venezuela (Darrow, 1989); Saudi Arabia (Boland and Gordon, 1989); Cuba (Fernandez, *et al.*, 1990); and India (reviewed by Cannham, 1989).

Of late, there are some new schemes of ET such as MOET (the use of multiple ovulation and embryo transfer) in combination with AI as a new breeding method for use in dairy cattle, which was for the first time been conceptualized by Nicholas (1979). Characterized by the formation of a central breeding herd (nucleus her) with extensive use of superovulation of donors selected among the heifers and young cows, MOET provides a more rapid rate of genetic improvement than that currently provided by AI. Another alternative is to employ the breeding improvement method known as open nucleus breeding scheme (ONBS). In the ONBS, a genetically superior nucleus herd or flock is established under controlled conditions where testing and genetic selection can be carried out (Holgers, 1990). This test group is first established by screening the base population for outstanding females. According to Jasiorowski (1990), the ONBS has the advantage of not requiring mass field recording and may be particularly applicable to livestock production in the developing countries. The two schemes, ONBS

combined with MOET, may offer the potential for greater progress in genetic improvement in cattle industry than the traditional well-established animals selection methods; hence permitting high quality breeding stock to be available for sale in larger numbers than was previously possible.

In developing countries, the greatest threats to genetic diversity appear to be increased by the use of AI and indiscriminate crossbreeding of indigenous breeds (Ruane, 1993). The intensification of farming in these countries can mean that indigenous breeds are in danger of being pushed to extinction because native farmers, in aiming for greater production, have employed exotic breeds such as Holsteins or Friesians. The application of *in vitro* fertilization (IVF) techniques can avoid such a problem. The practical application of IVF technology in the preservation of an endangered cattle breed has been described by Solti, *et al.* (1992), working with Hungarian Grey cattle.

The application IVF/ET technology as a means of upgrading the quality of cattle in developing countries may be one possibility worth exploring. As noted above, crossbreeding native cattle with exotic ones can raise serious problems. There are a number of scientific studies showing that *Bos taurus* x *Bos indicus* crossbreeds may have particular merit in combining the qualities of productivity with the ability to withstand the rigours of the tropical and subtropical environment. But, under conventional animal breeding schemes, however, such crossbreeds cannot be sustained beyond the first generation. In order to maintain the milking cow at 50% *Bos taurus*: 50% *Bos indicus* so as to exploit

heterosis, the use of IVF technology to produce embryos derived from European dairy cattle (like Holstein, Friesian, Jersey) and native bulls (zebu) may be one of the best solution (i.e., using ovaries from exotic breeds and zebu semen).

Lastly, though not least, ET is yet another possibility of exploiting developments in reproductive technologies, such as embryo sexing, splitting (demi-embryos), cloning, chimeric and transgenic animals production. Genetic engineering, for instance, could produce pharmaceutical products to increase livestock productivity such as growth hormone (to accelerate growth or to increase milk production); fertility hormone (to accelerate growth or to increase milk production); fertility hormones (to increase ovulation, conception, and oestrus synchronization); proteins that modulate immunity such as interferon and interleucon; and many others.

Despite the fact that current financial limitations offset our constant efforts to carry-forward many agricultural researches which are essentially meant to solve some basic problems impending national economic growth, it is high time that our government realize the importance of establishing a kind of problem-solving technologies through intensive research, rather than concentrating on social forces. Otherwise, one of the supposed benefits of a "late developer" is the opportunity to take advantages of technology that other have developed.

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