MULTIPLE OVULATION EMBRYO TRANSFER (MOET) IN DAIRY CATTLE IN GATTON

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ABSTRACT. Multiple ovulation and embryo transfer is one of the reproductive technologies which is important to increase animal production. In this case report, four cows were selected as donors while eleven cows were selected as recipients. Both donors and recipients had undergone the same procedures and steps for multiple ovulation and embryo transfer (MOET), such as cow preparation, synchronisation and where only donor cows follow the superovulation protocol. Cows were artificially inseminated and the embryos were flushed and graded. Out of four selected cows for donor, one of them was pregnant and only two cows actually produced the embryos. The recovery rate for the embryo collection was 70.1%, and from 13 embryos (including unfertilised ova), 84.6% of the embryos was classified as good quality and suitable for embryo transfer. The overall results showed that out of eight recipients, five cows were detected pregnant, a 62.5% pregnancy rate. The aim of this report is to describe the procedures as well as the factors that affect the successful of the MOET programme.

Keywords: multiple ovulation embryo transfer, estrus synchronization, superovulation, pregnancy rates

INTRODUCTION

Multiple ovulation and embryo transfer (MOET) can be defined as a process or steps in removing the fertilised eggs from a female donor and putting them in multiple surrogate recipients, who are not related genetically (Hasler, 2004; Jainudeen *et al.*, 2000). This technique can be carried out in a range of farm animals such as cattle, sheep, goat, buffalo and pig except in horses which are not able to be superovulated.

The MOET programme is widely carried out in cattle because it increases the production of offsprings significantly (Glen, 1977). There are a number of advantages of MOET in cattle; including improved and increased number of progeny, either male or female, from genetically superior donors. In addition, this kind of technique would allow the superior cow to produce a number of offsprings more than through normal reproduction. The MOET application can also lead to increasing the reproductive capability and ability of precious or valuable animals, and at same time increasing the percentage of genetic improvements of the herd (Critzer *et al.*, 1980). It can also be used to test for inherited defects of the bulls. By mating the bull with his superovulated daughters,

the recessive gene will be exhibited. These significant tests can save time and cost as the whole population need not be tested.

Furthermore, this process can be applied as treatment for infertile females due to disease, injury or ageing (Shenk *et al.,* 2006). Another advantage of MOET is, it could minimise the disease transmission risk geographically as well as from herd to herd, or from dam to calf as several diseases that exist in the mother would not be transmitted into the embryo.

Several factors have been identified which affects the increment of pregnancy rates and the success of MOET as described by Hasler (2001). As mentioned by Fry (2010), the factors affecting MOET may include nutrition, condition score of donor and recipient, stage of cycle, sensitivity to follicle stimulating hormone (FSH), follicle population, stress and bull effect.

Thus, the objective of this report is to provide the process and review the factors affecting the success of multiple ovulation embryo transfer in cows.

MATERIALS AND METHOD

Animal and management components of embryo transfer

Four dairy cows of Holsten Friesian breed were identified as donors and eleven cows were identified as recipients. The animals were adults of various ages, in good body condition with an average of 4 to 5 body score. The programme started at 9 am, on 4 April 2010, at the Gatton Dairy Unit, Australia. Prior to commencement of the programme, all animals were herded from the paddock and aligned into a cattle crush to begin the activity.

Oestrus synchronisation was done by trained technical staff using the injection of prostaglandins and inserting the controlled internal drug release (CIDRS), intravaginally, which contains 1.9 g progesterone per device. The hormone will prepare the uterus for reception of a fertilised ovum and suppresses the development of new graffian follicles (Michael, 2010). Figure 1 shows the time line of activity for the process of MOET.

During the next two days (6 April 2010, 9:00 am) both donors and recipients were injected with 5 ml gonadotrophin releasing hormone (GnRH) (intramuscular). Four days after that (10 April 2010) all the donors were injected with 4 ml follicular stimulating hormone (FSH) in the early morning (6:00 am) and late afternoon (6:00 pm). Heat detector (K-mars) was applied on the cows. All donors were injected again with FSH twice a day 3 ml (at 6:00 am and 6:00 pm, 11 April 2010); all recipients were injected with prostaglandin F2α (Lutalyse® 5 ml) at 12:00 pm. On 12 April 2010, all the donors were injected with FSH 2 ml and prostaglandin F2α (Lutalyse® 5 ml) in the morning (at 6:00 am) and then were injected again with the same hormones, FSH 2 ml and prostaglandin F2α (Lutalyse® 5 ml) in the afternoon (6:00 pm). The CIDRS were also removed from all the donors in the afternoon. The CIDRs were removed out at 12:00 pm. On 13 April 2010, the donors had the last injection of FSH 1 ml and prostaglandin F2α (Lutalyse 5 ml) twice a day (at 6:00 am and 6:00 pm). Heat observation was undertaken three times a day (at 6:00 am; 12:00 pm; 6:00 pm), for both the donors and the recipients. On 14

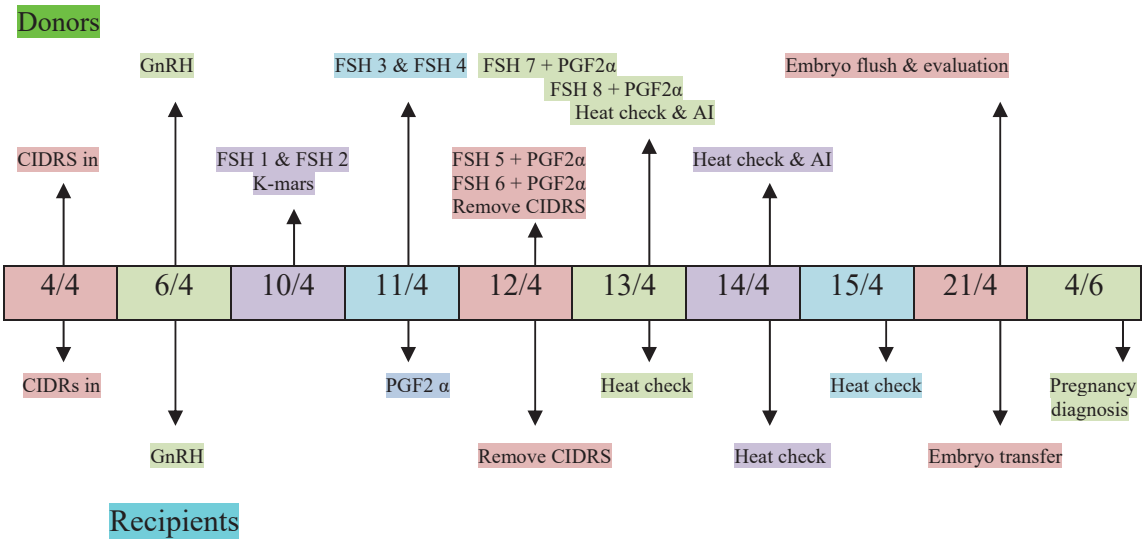

Figure 1. Time line of activity for the process of MOET.

April 2010, the donors and recipients were observed for their heat again three times a day. The observation of heat was repeated the next day.

Procedure and management of the embryo transfer was according to Fry (2010) and Hafez (2000). Artificial insemination was 6 to 12 hours after the cow was injected with FSH to produce superovulation. The donor cows were inseminated 2 to 3 times at 12 hour intervals, starting from 12 hours onset of standing heat. The procedure of artificial insemination was according to Fry (2010). After 8 days of oestrus (21 April 2010), the donor cows were placed in a constrict crush to non-surgically perform flushing of embryos. The embryo recovery, handling and transfer was based on Fry (2010) and Michael (2010). The detection of pregnancy in all recipients was by ultrasound.

RESULTS AND DISCUSSION

Success rate of estrus synchronization

Three donor cows out of four showed signs of standing heat at different times. The first donor cow (ID 391) showed signs on day 9 after removal of CIDRS. It was inseminated four times. The second donor cow showed signs of standing heat on day 11 (ID 415) after 3 artificial inseminations. The third donor cow showed standing heat on day 13 (ID 871) after one insemination. The fourth cow did not show any sign of oestrus, and hence, probably pregnant. A rectal palpation confirmed that it was pregnant.

For recipient cows, eight cows out of eleven showed signs of standing heat on day 10 and another two cows on day 11. One recipient cow (ID 674) was removed from the programme because it was suspected pregnant. Overall results showed that more

than 50%, both donor and recipient cows were having oestrous synchronisation with slightly differing periods approximately 12 to 24 hours. The response of FSH as the supporting hormone for super ovulation is varied among the donors. This is indicated by the various time durations of their heat even though the FSH was injected twice a day to stimulate follicle growth.

Table 1 shows the superovulation response in each donor animal.

Number and quality of embryo in the table showed that each individual embryo was located and evaluated using a microscope. The evaluation was based on the quality of the embryo and classified numerically as:-

1. Regularity of shape of the embryo
2. Compactness of the blastomeres (the dividing cells within the boundaries of the embryo)
3. Variation in cell size
4. Color and texture of the cytoplasm (the fluid within the cell wall)
5. Overall diameter of the embryo
6. Presence of extruded cells
7. Regularity of the zona pelucida (the protective layer of protein and single-celled embryo)
8. Presence of vesicles (small bubble-like structures in the cytoplasm)

The embryos were evaluated according to their stage of development:

Stage1: Unfertilized
Stage2: 2 to 12 cell
Stage3: Early morula
Stage4: Morula
Stage5: Early blastocyst
Stage6: Blastocyst
Stage7: Expanded blastocyst
Stage8: Hatched blastocyst
Stage9: Expanding hatched blastocyst

The classification of the embryos was based on these criteria:

Grade1: excellent or good
Grade2: fair
Grade3: poor
Grade4: dead or degenerating

Recipients for 669, 666, 660 and 665, the description of class of embryo can be defined as the development of morula and graded as fair. While for the recipients of 662, the development was still morula but was graded as poor. Recipients for 670, 667 and 668, the development of embryo were on early blastocyst with fair classification.

Table 1. Superovulation response in each donor.

Number of donor	Date on heat	Hours after CIDRS out	Number of embryos	Number of CL
391	13 April 2010 – morning	12 hours	9 good, 1 bad	11
415	13 April 2010 – afternoon	36 hours	2 good, 1 unfertilised	6
871	14 April 2010 – mid day	54 hours	No embryo	No CL detected
467	Pregnancy detected			

Table 2. Number and quality of embryo.

Recipients	Donors Embryo	Descriptive Term of Embryo	Class of Embryo Stage (Grade)	Left or Right Horn
669	415 (1)	Good compact morula	4 (2)	Left
666	415 (2)	Good compact morula	4 (2)	Left
662	391 (3)	Fair compact morula	4 (3)	Right
670	391 (4)	Poor early blastocyst	5 (2)	Right
667	391 (5)	Poor early blastocyst	5 (2)	Right
660	391 (6)	Good compact morula	4 (2)	Right
665	391 (7)	Good compact morula	4 (2)	Right
668	391 (8)	Poor early blastocyst	5 (2)	Right
661 & 663	No Embryo Transferred	No CL		
674	Removed	Not in MOET program		

Table 3. Pregnancy rate.

ID RECIPIENT	DESCRIPTIVE RESULT	RATE
665	Pregnant Age approximately 7 weeks after ET White K-Mars Much fluid in the uterine horn	12.5 %
669	Not pregnant Red K-Mars Follicle 16 mm	
670	Pregnant Red K-Mars Much fluid in the uterine horn	25 %
662	Pregnant White k-mars	37.5%
666	Not pregnant Red K-Mars Big corpus luteum retained, in cycling Non-pregnancy cycle	
660	Red k-mars Pregnant CL develop nicely with follicles around	50%
668	Not pregnant Red k-mars Corpus luteum retained at one side Next follicle ovulate 2 Large follicle	
667	Pregnant 3 to 4 months White K-Mars Much fluid in the uterine horn	62.5%
Total	5/8 recipients cow found pregnant Rate: 62.5 % Conclusion: success	

Based on the pregnancy diagnosis which was undertaken on 4 June 2010, the descriptive results showed that 3 recipients (669, 666 and 668) were detected not pregnant, while another 5 recipients (665,670, 662, 660 and 667) were successfully pregnant. Thus, overall pregnancy rate was 62.5 %.

The overall result on pregnancy rates for this programme was 62.5%. Thus, this programme was classified as successful. Based on a previous study by Hasler (2001), the pregnancy rates for two (2) different locations, were 68.3% and 77.1% respectively for fresh embryo. While for frozen thawed embryo, a study in The Netherlands reported the pregnancy rates as 56.1%. In two other studies done in The United States, the rates were 58.4% and 68.7% respectively.

Oestrus synchronisation between donors and recipients were simultaneous within the same range period. As suggested by Wright (1981), oestrus synchronisation is one of the most important factors in embryo transfer. Almost all commercial facilities that performed the procedure showed a significant decrease in pregnancy rates where recipient oestrus was detected 12 hours after the donor's (Wright, 1981).

It was remarkable that embryo from one donor (ID 415) resulted in two non-pregnant recipients (ID 666 and 668) when its embryos were transferred to these recipients. This condition could be due to delay in standing heat, which was 36 hours after CIDRS was removed. Hence, fewer embryos were produced and only two embryos were found appropriate to be transferred to recipients.

On the other hand, embryos from the other donor (ID 391) resulted in five pregnant recipients. The possible reason for success may be that the embryos transferred were of good category. Only one embryo was classified as poor early blastocyst and because of that, it did not lead to pregnancy in one recipient.

Embryo transfer is not only a process of transferring an embryo from donor to recipient but includes several steps in the process to determine the success of the procedure. As suggested by Hasler J.F. (2004), there are several factors that influence the success of embryo transfer:

i. Superovulation: approximately 20% of donors produce no functional embryo and over three rounds of superovulation, a range of 19% to 58% of donors failed to produce a pregnancy. However, there is an increment of embryo production per donor on a per unit time basis through the use of intravaginal or subcutaneous progesterone-releasing devices.
ii. Quality of embryo also plays an important role in determining the successful of pregnancy rates. Evaluation of embryo is now well standardised by International Embryo Transfer Society according to the stage of development and quality based definitions. Several literatures have suggested that embryo grading is useful for predicting pregnancy rate.
iii. Embryo handling and freezing are very complicated and often influence the pregnancy result. The usage of frozen embryos results in a decline

in pregnancy rates compared to fresh embryos at approximately 10 to 20 percent. As claimed by Hasler (2001), the usage of high quality fresh embryos transferred into surrogate recipients can achieve an average pregnancy rate of up to 80%. For this programme, only fresh embryos were used. However, the result was not as high as suggested.

iv. Age of embryo also can affect the result of embryo transfer. Survival rates following the transfer at Day 3 and Day 4 embryos are lower than the transfer of later-stage embryos. Embryo developmental stage should be matched with recipient cycle stage even though not always possible but it could be advantageous and beneficial.

There was a source of error during implementation of the procedure. Its CIDRS device was unfastened in one of the recipients which resulted in the loss of the device and could not be detected. This recipient cow was not synchronised with the other recipients and found pregnant. In addition, observation of heat also could be confused due to the misunderstanding of behavioural and physical signs of oestrus.

This MOET programme can be improved by good preparation of both donors and recipients in terms of selection and history. This study recommends to have a record book to note down oestrus signs. Also, a better understanding of the physiology and behavior of cows would be beneficial in misunderstanding the signs of oestrus.

If the programme needs to be implemented and continued in Malaysia, the factors that have been discussed should be taken into consideration to ensure the success of the MOET programme.

CONCLUSION

Embryo transfer is the procedure of transferring an embryo from a donor to a recipient by a series of steps that are dependent on factors associated with the embryo, the donor and recipient and interaction among factors of the embryo and recipient. Several considerations should be taken into account when deciding to implement an embryo transfer such as the preparation of donor and recipient, the selection of embryo to be transferred into recipient and the requirement for close synchrony of oestrus between the donor and recipient. It is very important to ensure that successful pregnancies of recipients are at higher rates beneficial to the animal industry.

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