THE STUDY OF MULTIPLE OVULATION AND EMBRYO TRANSFER (MOET) ON THE BASE OF OESTRUS SYNCHRONISATION USING GnRH, FSH and PGF2α

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Abstrak: MOET merupakan suatu program perbaikan mutu genetic untuk mengurangi keterbatasan ternak betina dalam menghasilkan bibit kualitas unggul.Proyek ini bertujuan mensinkronkan siklus estrus antara donor dan resipien dengan menggunakan hormone GnRH, PGF2£, and FSH, hingga ternak resipien siap untuk menerima transfer embrio pada saat yang tepat. Ternak percobaan yang digunakan adalah ternak perah, yang terdiri dari donor 4 ekor sapi laktasi dan resipien 8 ekor masing – masing dengan score (body condition score) 4-5. Percobaan dilakukan di laboratorium lapangan ternak perah, University of Queensland, Australia.Proyek ini dilakukan dalam beberapa tahap, yaitu: sinkronisasi estrus, super ovulasi, inseminasi buatan, perbaikan embrio, evaluasi embrio, perlindungan dan pemeliharaan embrio, transfer embrio dan diagnose kebuntingan.Hasil percobaan menunjukkan bahwa 2 dari 4 ternak donor memiliki kualitas embrio yang bagus (good compact morula), dengan jumlah masing-masing 4 embrio. Proses sinkronisasi estrus antara donor dan resipien relative sama, dimana dengan rata-rata 12 jam – 36 jam setelah CIDR dicabut ternak menunjukkan tanda-tanda estrus. Angka kebuntingan 62.5% dari total jumlah ternak resipien. Berdasarkan hasil tersebut, maka skema yang dicobakan dapat diaplikasikan dalam program MOET

Kata Kunci: sinkronisasi, estrus, embrio-tranfer

INTRODUCTION

Improving livestock production through animal breeding strategies is mainly for the purpose of increasing efficiency., which can be achieved through MOET. The technique of multiple ovulation and embryo transfer (MOET) has shown some results that could reduce these limitations on the female side. MOET program consist of some steps, firstly as the base is synchronising the oestrus cycle between the donors and the recipients, then followed by super ovulation, artificial insemination, embryo recovery, embryo evaluation, embryo cryopreservation, embryo transfer and finally pregnancy diagnosis.

This experiment will investigate the scheme in synchronising the oestrus cycle of both donors and recipient by using the hormones: GnRH, PGF2£, and FSH, in order to efficiently achieved the successful of MOET which can be observed through the percentage of good quality embryos and the pregnancy rate.

The Advantages of MOET in Cattle

In several simulation models it was argued that MOET breeding schemes are superior to conventional breeding strategies for dairy cattle using artificial insemination (AI) (e.g. (Nicholas & Smith 1983).
In the mid 198Os, several MOET schemes were initiated in practice. From an embryo technology point of view, these schemes represent a challenge as they will provide the ultimate proof of the practical usefulness of the techniques of super ovulation and embryo transfer in cattle breeding. MOET offers a unique possibility for collecting and thoroughly analysing information concerning super ovulation and embryo transfer in a system where the donors and recipients are under strict control (Callesen et al. 1996).

The benefits of MOET program has been revealed in some previous studies which are: Increase rate of genetic gain or genetic response in the unit establishment; Decrease generation interval significantly, “from conventional 7 years to 3” (Tierney QDPI in Collard 2003); Enhance female selection intensities in the herds group, in this case increase female reproductive rates (Gordon 2004), through the process of super ovulation and embryo transfer (Weigel 2001), (Penna 2003).

Gordon further suggested that MOET could significantly increase the rate of genetic improvement in any species in which the natural reproductive rate is low. Moreover, if high rate of ET could be achieved, the rate of genetic improvement could even be doubled (Gordon 2004). Penna concluded the advantages of MOET that mainly speed the selection. As it has been observed that younger bulls have more and better semen quality compared to older bulls and also have longer reproduction life (Penna 2003).

However, there was some limitations in MOET. It was reported that the response to FSH might be expected approximately 70%, the response of the ovarian was highly varied (0-80 CL’s), FSH dose can not be determined accurately, unfertilised ova due to the poor sperm management, cystic follicles, refractory to stimulation, and infertility (Fry n.d.).

Conversely, by concerning the factors that can contribute to the success of MOET, such problems can be avoided, such as through synchronising the oestrus cycle as the base, then the appropriate time for artificial insemination and the techniques to achieve more number of good quality embryos, which hence will influence the pregnancy rate.

The physiology behind all facets of the MOET program

Preparation of the donors

- Oestrus synchronisation

Oestrus detection should be done accurately to both donors and recipients. Oestrus characteristics can be recognised through visual observation, which can be undertaken in early morning or late afternoon. The cows which are on heat will show the obvious mucosa on their vulva and mounting behaviour, restless behaviour, etc. Oestrus behaviour can be observed at once after prostaglandin treatment (oestrus synchronisation) (Seidel & Seidel 1991). The requirements are: fertile heifers and cows on an adequate nutrition program; high quality semen for AI; healthy, aggressive, and fertile bulls for synchronized natural breeding (Deutscher n.d.).

- Superovulation

The requirements of the donors are high milking ability, high growth rate, high meat quality, and outstanding reproductive capacity (Fry n.d.). The fertile donors which are used at least 2 months post-partum (Seidel 1991).

- Artificial insemination

The bulls should have more and high quality semen(superior genetic), physically in good health, and good condition of reproductive tracts. Both factors physic and genetic should be considered in order to get high quality offsprings.

- Embryo recovery

The donors should have high genetic quality and the ability to produce large number of good embryos. The donors are flushed through non-surgical procedures approximately a week after oestrus, due to the proper embryo stage (compact morula and blastocyst). It is expectred that four to six transferable embryos will be recovered (Gordon 2004), though it is possible to be more.

- Termination of unrecovered embryos

In order to stimulate the new-oestrus cycle, the donors will be injected with PGF2a, for two times at one week interval. Rectal palpation or ultrasonography technique can be applied 40-50 days after flushing, to observed the result.

Preparation of the recipients

- Oestrus synchronisation

Yearling heifers and mature cows in good body condition; and gaining weight prior to treatment are the best candidates for successful synchronization results; Twoyear-old heifers are usually poor candidates because they are slow in cycling and rebreeding after their first calf; Yearling heifers need to reach target weights (650-750 pounds depending on

breed) for a high percentage to be cycling before the breeding season; Cows should generally be 45 days postcalving before treatment is started. The majority of synchronized females will calve during a two-week period with a maximum of 20 percent calving in one day (Deutscher n.d.).
Oestrus observation in recipients can be done in early morning or late afternoon (Seidel 1991). Generally, the oestrus cows will just stand while mounted by others. However, it is also possible that the cows that do the mounting also on oestrus.

- Embryo transfer

Healthy and fertile heifers should be treated in non-pregnant condition, in order to wait for the embryo transfer.

- Pregnancy diagnosis

At about day 26 of pregnancy in heifers and day 28 in cows, pregnancy can be diagnosed accurately under field conditions by ultrasonography or even earlier in very skilled hands (Kastelic et al., 1988). Seidel recommended to applied rectal palpation at 45–60 days of gestation and confirm this with another palpation one month later (Seidel 1991).

MATERIALS AND METHODS

Materials

In this trial, it was used 4 lactating dairy cows as donors and 8 cows as the recipients. The animals were at multiple age, adults, have good body condition with average of 4 to 5 body score. The trial was done in dairy farm, University of Queensland, Gatton. It was started at 9 a.m, on 4 April 2010. All animals selected were gathered from paddock and force to enter the crush each time before beginning the program.
Hormones used .were GnRH, PGF2α, FSH, and progesterone in CIDR device.

Methods:

Oestrus synchronisation program for donors and recipients.

- The acitivity was started on Sunday 4 April at 9.00 am in the dairy Gatton. CIDR(Controlled Internal Drug Release) is sterilized then inserted into the cervix in both donors and recipients on day 0, to avoid pregnancy.
- CIDRS contains Progesterone 1.9g per device. The hormone will prepare the uterus for reception of a fertilized ovum and suppress the development of new graffian follicles.

  The scheme introduced in this experiment started from oestrus synchronisation between the donors and the recipients until the embryo transferred is described below:

Table 1. Time Interval of MOET experiment

Donors								
4/Apr CIDR in	6/Apr GnRH Injected	10/Apr FSH: 6a.m. Heat detector; 6p.m FSH: 6p.m.	11/ Apr FSH:6a.m FSH:6 p.m	12/Apr FSH+ PGF2α : 6a.m FSH+ PGF2α : 6p.m CIDR out	13/Apr FSH+ PGF2α : 6a.m FSH+ PGF2α : 6p.m on heat AI	14/Apr On heat AI	21/Apr Embryo flush+ evaluation	
Recipients								
4/Apr CIDR in	6/Apr GnRH **injected**		11/ Apr PGF2α :12p.m	12/Apr CIDR out	13/Apr On heat	14/Apr On heat	21/Apr Embryo transfer	4/Jun Pregnancy diagnosis

Embryo recovery procedures

The embryos were recovered by non surgical flushing of the uterus.Embryos were recovered from those super ovulated donors through trans vaginal insertion of a catheter into the uterus

Embryo cryopreservation procedures

Embryos were collected 6 to 8 days post oestrus by repeated flushing of Dulbecco's phosphate buffered saline (PBS) containing 2% heat inactivated foetal calf serum (FCS) through a Foley catheter in the uterus (Wright 1981). The embryos are then placed in a concentrated glycerol solution: 1.4 M in Phosphate Bovine Serum/PBS supplemented with Bovine Serum Albumin/BSA) as the buffer medium, at room temperature. The embryo is allowed to equilibrate for a 20 minute period. One embryo is loaded into 0.25 or 0.5 ml French straw.

Embryo transfer into recipients

Embryos which had been collected from the two donors were freshly transferred to eight recipients 8 days after oestrus was detected.

RESULTS

Report on success rate of oestrus synchronisation and embryo transfer

The success rate of oestrus synchronisation can be observed below:

Table 2. Oestrus synchronisation in the donors

Number of donor	Date on heat	Hours after CIDRS out
391	13 April 2010-morning	12 hours
415	13 April 2010-afternoon	36 hours
871	14 April 2010-mid day	54 hours
467	Pregnancy detected	

Three donor cows out of four experienced standing heat with different time. ID 415 was inseminated four times. The donor cow was firstly on heat was ID 391, ID 415 and ID 871, with the time of heat after CIDR out was 12 hour, 36 hour, and 54 hour, respectively.

Of the recipient cows, eight cows were experienced standing heat at same day, which were averagely 12 hours and 36 hours. Overall, more than 50% of both donor and recipient cows had oestrous synchronisation with slightly different period.

AIwas done 6 – 12 hours after the cows were injected FSH and the releasing of CIDR to produce super ovulation. The cows were inseminated two to three times with 12 hours intervals starting from 12 hours onset of standing heat.

There are 50% of the donors (2 out of four cows) were detected to have good quality embryos which are in grade 4 (good compact morula); each of them had four good embryos that were then transferred to eight recipients.

The response of FSH as the supporting hormone for super ovulation is varied among the donors. This was indicated through the various time duration of their heat (Table 1), even though the FSH was injected twice a day to stimulate the follicle growth.

Report on the pregnancy rate

Based on the pregnancy diagnosis, the pregnancy rate can be described as follows:

Table 3. The description of pregnancy diagnosis in the recipients

ID RECIPIENT	ID DONOR	DESCRIPTIVE
665	391	Pregnant
669	415	Not pregnant
670	391	Pregnant
662	391	Pregnant
666	415	Not pregnant
660	391	Pregnant
668	391	Not pregnant
667	391	Pregnant
Total		5/8 recipients cow found pregnant Rate: 62.5 % Conclusion: success

The table showed that pregnancy rate is 62.5% which means that more recipients were detected pregnant. More cows were pregnant from the embryo transferred from donor 391.

DISCUSSIONS

MOET trial in Gatton that use the oestrus synchronisation scheme with the interval as described above, showed a successful oestrus synchronisation of both donors and recipients and more good quality embryos with the pregnancy rate of 62.5% recipients, which means that the scheme can be applied in the process of MOET because during the interval of two months of the cows can show pregnancy again, through the embryo transfer. This is probably due to the good synchronisation of both the donors and the recipients.

As the support, a report indicated that a large number of embryo transfers performed by a commercial facility and showed a significant decrease in pregnancy rate where recipients were used that were in oestrus 12 hours after the donor cow (Wright 1981), which means not in good synchrony. Moreover, Sreenan and Diskin (Sreenan & Diskin 1987) reviewed that between 19% and 58% of donors were not successfully

There are some possible factors that can influence the pregnancy rate, which can be from the donor, the embryo itself, the recipient, or the freezing and thawing process. Sreenan and Diskin (Sreenan & Diskin 1987) suggested that the possible cause might be: 1). Donor effect on the survival rates of post transfer embryo; 2). Embryo age, where matching of embryo developmental stage with recipient cycle stage appears to be advantageous though not always possible; 3). Embryo quality, where poor qua1ity embryos should first be cultured for a short period to better determine their quality and then transferred to a synchronous environment; 4). Donor-recipient synchrony, where it will be advantageous if the recipient is in oestrus in advance of the donor due to the changing in uterine environment.

To support, Gordon (Gordon 2004) stated that some factors that contribute to the success of embryo transfer are skills and experience of the ET operator; selection and management of the recipients; good synchrony of oestrus between donors and recipients; quality of embryos which are transferred; and the methods which are applied in embryo handling and transfer. In addition, it is better to transfer fresh embryos rather than transferring frozen-thawed embryos because it will cause the pregnancy rate 10% **below** using fresh embryos (Gordon 2004).

CONCLUSION

1. The success of embryo transfer depends on factors associated with the embryo, the recipient or an interaction among factors of the embryo and recipient.
2. It is important to consider the requirement for close synchrony of oestrus between the donor and recipient cows.

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