RESEARCH PAPER
AL-ANBAR JOURNAL OF VETERINARY SCIENCES
Vol. 12 issue: 1 L- ISSN :1999-6527

The influence of Multiple Ovulation and Nonsurgical Embryo Transfer on pregnancy rate in Holstein Friesian Heifers

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Doi: https://doi.org/10.37940/AJVS.2019.12.1.14

Abstract

This study was carried out by selecting a total number of 17 Holstein Friesian cows divided into two main groups, the first group (4) cows as donors used to take the embryos from after artificial insemination and the second group (13) heifers cows as recipients for embryos. The same techniques were used on donor and recipient cows for the purpose of hormonal preparation in terms of instance synchronization, preparing recipient cows and superovulation for donor cows. Artificial insemination were done in field, and a nonsurgical flushing procedure embryos applied then graded. Donors reflected a good response to superovulation hormonal treatment. The total number of transferable embryos was 13 from four donors. Evaluation of all embryos was excellent quality. The pregnancy rate results for recipient cows was 61.5%. This result is an acceptable as compared to previous studies due to many factors, the study aim was taking into account all these factors, that can have an influence on these programs to obtain genetically improved animals in a shorter time and lower costs.

تأثير فرط الاباضة ونقل الأجنة بالطريقة غير الجراحية في نسبة الحمل لسلالة أبقار الهولشتاين فريزيان

الخلاصة

اجريت هذه الدراسة عن طريق اختيار (17) بقرة هولشتاين فريزيان قسمت إلى مجموعتين رئيسيتين, الأولى (4) ابقار مانحة تم استعمالها لأخذ الأجنة منها بعد التلقيح الصناعي والمجموعة الثانية (13) بقرة مستقبلة لهذه الأجنة. تم استخدام التقنيات ذاتها على الابقار المانحة والمستقبلة لغرض تهيئتها هرمونيا من ناحية تزامن الشبق، وإعداد الأبقار المستقبلة وفرط الاباضة للأبقار المانحة اذ تم اجراء عملية التلقيح الصناعي في الحقل، و بعد ذلك تمت عملية نقل الاجنة بالطريقة غير الجراحية بعد اجراء عملية التقييم. الابقار المانحة اظهرت استجابة جيدة لعملية فرط الاباضة باستعمال الهرمونات. ان مجموع العدد الكلي للأجنة المستخرجة من الابقار المانحة كان (13) من مجموع (4) أبقار، كان تقييم جميع الأجنة ممتاز من حيث النوعية، ان النسبة المئوية للحمل في الأبقار المستقبلة كانت 61.5٪, والتي تعتبر نتيجة مقبولة عند مقارنتها بالدراسات السابقة وذلك لتأثر هذا النوع من البرامج بالعديد من العوامل التي تناولتها هذه الدراسة بهدف الحصول على حيوانات محسنة وراثيا في وقت أقصر وتكلفة أقل، باستعمال برامج فرط الاباضة ونقل الأجنة (MOET), مع الأخذ بعين الاعتبار جميع العوامل التي يمكن أن يكون لها تأثير على هذه البرامج.

الكلمات المفتاحية : فرط الاباضة ، نقل الاجنة ، توحيد الشبق ، نسبة الحمل

KeyWords : Multiple ovulation and embryo transfer, estrus synchronization, pregnancy rate, superovulation

Introduction

Recently, many studies have been carried out to improve the reproduction in domestic animals. Multiple ovulation and embryo transfer is one of Assisted Reproductive Techniques (ART), that have been widely applied in different animal species.

The definition of Multiple ovulation and embryo transfer (MOET) stands for several steps that start by collection of fertilised ova from donors and transfer them to recipients (1,2). Embryo transfer has become the most powerful tool animal breeders which have been developed meanwhile Artificial Insemination (AI). Multiple ovulation and embryo transfer (MOET) was used originally to increase the numbers of embryos, from genetically elite cows for short time periods in a range of farm animals such as cattle, sheep, goat, buffalo and pig excluding horses because it do not have capability for superovulation . At the present time, MOET is also used as a technique to decrease the negative effects of heat stress on fertility and pregnancy rate after embryo transfer in dairy cow recipients (3).The use of MOET scheme has been reported for many years; in particular it has been used on dams of bulls in order to be used in artificial insemination (AI) procedures(4). Several studies has been reported on the use of multiple ovulation and embryo transfer (MOET) in cattle especially in heifers. MOET program has the possibility to increase the number of offspring from genetically superior females at specific age. So that MOET can increase the selection intensity between cows in order to get the desired donor animals. Besides, the use of MOET can reduce the generation intervals in the paths from dam to daughter and dam to son (5).

Additionally, the rate of genetic improvement and selection intensity of females can be increased by the use of MOET scheme for potential reproductive increase. Besides, it can be used to breed replacements from younger females; therefore, it can reduce the generation interval and speed up genetic improvements. The use of MOET program is also important for animal welfare considerations that can use non-surgical rather than surgical procedures. Moreover, the use of MOET can allow preselection of particular gender through sex determination in order to increase the number of desired calves from a limited numbers of embryos (6). Furthermore, using MOET program gives the opportunity to select females that may have the ability to produce large families. Besides, the bull offspring can be evaluated by selecting the desired embryos to improve the biological and economic efficiency of beef and dairy cattle production systems. Good genetic traits from valuable animals can be preserved for a long time, which can lead to greater reliance on a few breeds for embryo transfer. In addition, MOET program can allow large scale progeny testing in bull expansion of small populations of valuable cows. Particularly, it enables the genetically superior cows to produce a high numbers of calves (7). The reason behind using multiple ovulation and embryo transfer (MOET) programs in cattle is to obtain the successful production of offspring from cattle with high genetic merit. Prediction of successful MOET programs is desirable for genetic improvement and to reduce costs (financial welfare) by saving hormone treatments and reducing waste of high-value semen and embryos (8).Fry R. (9) stated that MOET programs can be carried out for treatment purpose for females infertility due to disease, injury or aging. Additionally, decreasing the danger of geographically diseases transmission as well as from herd to herd, or from dam to calf. As a consequence, these diseases will not have the potential to be transmitted into the embryo. The author also indicated that factors influencing MOET programs comprise, nutrition, body condition score of donor and recipient, stage of cycle, sensitivity to follicle stimulating hormone (FSH), follicle population, stress and bull effect. Therefore, the study aimed to show the influence of different factors on MOET programs.

Materials and methods

1. Experimental animals:

The total number of animals that were used in this MOET program was 17 cows of Holstein Friesian breed. Four cows were used as donors to collect the embryos from. Whereas, 13 heifers were used to be a recipients for these embryos. All cows maintain a good body condition score and healthy condition in order to have the best conception rate. All recipient heifers were housed in the same pen with the same management at the dairy cattle station in Gatton campus, University of Queensland, Australia. Similarly, donors were housed in a separated pen.

2. Oestrus synchronisation program for donors and recipients:

MOET program was carried out within approximately 34 days at the dairy station at Gatton campus, University of Queensland. Prior to the superovulation treatment, all donors were injected with prostaglandin twice (ESTROMILL, Troy Ilium Company, Australia) with 11 days interval. Donors should be synchronized for estrus before the superovulation program commence (took 3 days). In order to have all cows coming on heat at the same time, CIDR (controlled intravaginal drug release) was used for oestrus synchronisation techniques. Insertion of CIDR for all cows was carried out on the fourth day of MOET program. The CIDR was removed from all cows after seven days of the insertion with an injection of prostaglandin (PGF2∝). Three days later, cows entered proestrus and estrus cycle. In addition, heat detectors (Kamar® heat mount detectors) were used for all cows on the epidural part of their bodies, and observation for heat signs commenced within three days (72 hours).

3. Superovulation program:

The injection of follicle-stimulating hormone (FSH), Folltropin 20 ml (Vetoquinol, France) was done for superovulation the donors from the day 10 after estrus. This procedure was carried out by injection of eight reducing doses of FSH (from 4ml in the first day to 1ml in the fourth day) over 3 to 4 days with a 12 hours intervals between the A.M. and P.M. injections. Besides, the injection of FSH was associated with a two doses of prostaglandin (PGF2∝) administerd at the fifth and sixth day of FSH in order to synchronise the donors' oestrus. After 52 hours of PGF2∝ injection, all donors showed signs of oestrus behaviour about 2 days later, allowing 4 days from the start of gonadotropin to onset of estrus.

4. Artificial insemination procedure:

Cows were inseminated after 12 hours of heat by using semen from a fertile bull. Generally, cows that showed heat at the morning should be inseminated in the afternoon, and cows that showed heat at the evening should be inseminated in the next morning.

5. Embryo recovery procedures:

One week after artificial insemination (AI), all donors were prepared for embryos recovery. A nonsurgical flushing procedure was used for donors, indicated by Fry R.(9) and one transferable embryo was recovered. Prior to embryos recovery, each donor cow treated by using local epidural anesthesia with 4ml lidocaine (Lignocaine injectable 100ml, Troy Ilium Company, Australia) and the vulva was cleaned before inserting the catheter. After that, A three-way Foley catheter (Flush catheter, Minitube, Germany)was used to recover the embryos through the cervix with the use of ViGro complete flush solution (2L) pack by Bioniche (400ml of phosphate-buffered saline (PBS) for each horn). The cuff is inflated, and the uterine horn irrigation started. After finishing the first horn, the same procedure was repeated on the second horn. The fluid used for recovery was collected through a filter (Embryo Collection Filters, Minitube, Germany). In addition, all donors were injected with PGF2∝ after the embryos recovered in order to terminate unrecovered embryos and prevent pregnancy.

6. Embryo evaluation procedures:

After collecting the embryos from the donors, all embryos was kept in phosphate-buffered saline PBS (ViGro Holding Plus 50ml, Bioniche) solution at room temperature, evaluation of embryos was done under microscope by counting the cells, number of extruded cells and evaluating the embryos (healthy ZP the quality embryo) according to grade and quality criteria of IETS.

7. Embryo transfer into recipients:

Non-surgical embryo transfer technique was used which involved the use of embryo transfer pipettes. The recipients were restrained in crushes. The embryos were loaded in 0.25 ml straw (Minitube, Germany), which was sterile, and between at least two air bubbles and the straws were loaded in the embryo transfer pipette. It was ensured that the straw engaged to the sheath tightly in order to avoid leakage. The sheath was coated with sterile, non-toxic obstetrical lubricant and the sheathed pipette was passed through the vulva up to OS cervix. The embryos were placed slowly and firmly in the uterine horn adjacent to the ovary with active CL by passing the pipette through the cervix, very similar to artificial insemination.

8.Pregnancy diagnosis:

Ultrasonography (USG) was used to diagnose the pregnancy for the recipient cows after approximately six weeks of transferring the embryos to the recipients (7 weeks after AI). The device consists of finger-mounted device connected to split screen. The finger-mounted was inserted through the rectum to monitor the entire corpus luteum (CL). Images of the uterine horn were displayed on the screen to examine whether the cow is pregnant or not. Images were taken and recorded for all recipient cows.

Results

1.Success rate of oestrus synchronisation :

After the last injection of PGF2∝, all donors came on heat within 34-52 hours. Heat detection of recipients was carried out by using heat mount detectors (Kamar, United states), which was very useful, and all cows got red marks on their bodies after 72 hours (three days) of PGF2∝ injection. In addition, All recipients cows came on heat within 24-54 hours.

2. Superovulation response in donors with the number and quality of embryos:

The superovulation rate was acceptable in this program, and all donors reflected a good response to superovulation treatment. The total number of transferable embryos was 13 from four donors. The quality of embryos classified as an excellent (Grade1), however, only one embryo classified as fair quality (Grade 3). Besides, the majority of embryos were in stage 4 (compact Morula), two of them were in stage 5 (early blastocyst) and two others were in stage 6 (blastocyst).

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Table (1). Embryo Evaluation (Adapted from Richard Fry 2010)

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

Fresh embryos were transferred into the recipient cows, and cryopreservation procedure has not been done.

Table (2) Superovulation response in each donor

Donor No:	embryo	ETS recipient-embryo stage/grade
551	3	213(4/1),193(4/1),180(4/1)
1159	5	107(4/1),205(5/1),164(5/1),189(6/1),699(6/1)
490	4 (1 deg.)	131(4/1), 110(4/1),186(4/3)
503	2	177(4/1), 126(4/1)

Table (3) The results of pregnancy diagnosis after 7 weeks of the embryo transfer

Donors	Straw	Recipients	The uterine horn	Pregnancy diagnosis result
1159	1	107	Right	Pregnant
	2	205	Left	Pregnant
	3	164	Left	Pregnant
	4	189	Left	Pregnant
	5	699	Right	Pregnant
503	6	177	Right	Non-Pregnant or lost her pregnancy (need to be rechecked)
	7	126	Right	Non-pregnant (regressing CL)
490	8	130	Left	Non-pregnant
	9	110	Left	Pregnant
	10	186	Left	Non-Pregnant
551	11	213	Right	Non-Pregnant
	12	193	Right	Pregnant
	13	180	Right	Pregnant

3. Pregnancy rate:

The success of pregnancy rate in MOET programs is normally between 50-70%. Besides, embryos were transferred to 13 recipient cows. The pregnancy diagnosis was carried out with ultrasonography seven weeks after transferring the embryos to the recipients' cows. The pregnancy rate was approximately 61.5% (8 pregnant cows out of 13 cows). The table below illustrate the numbers of pregnant and non-pregnant cows.

Discussion

The overall results of the current study regarding pregnancy rate was 61.5%. (8 out of 13) with taking into account that this MOET program was carried out as a part of university study and not for commercial purposes. The average pregnancy rates that were obtained in previous MOET programs ranged between 50-70% depending upon whether fresh or frozen/thawed embryos were used and whether surgical or nonsurgical transfer procedure were used (4, 5). In addition, Thiber & Nibar (10) stated that pregnancy rate ranged between 60-70% is acceptable in MOET programms. These previous studies support the results of our study. Therefore, this programme was classified as successful. Moreover, establishment of the Genus MOET nucleus herd, number of transferred embryos and number and percentage of pregnancy cows was reported between 1987 and 1990 by (11). The author indicated that pregnancy rates in 1987, 1988, 1989, 1990 were 65.4%, 62.8, 54.9 and 59.4 respectively. Hasler (12) carried out a study to examin the pregnancy rate following embryo transfer in Holstein cows and heifers by the use surgical and nonsurgical procedures over 20 years period. The total pregnancy rates in the first program with surgical and nonsurgical embryo transfer using fresh embryos were 71.0% and 46.9%, while the pregnancy rates in the second program with the use of surgical and nonsurgical transfers using fresh embryo were 71.8% and 54.1% respectively. Furthermore, a study by Bajagai, Y. S. (13) indicated that pregnancy rate was 62.5% (5 out of 8) after 45 days of non surgical embryo transfer in Holstein Friesian cattle. The author also stated that the numbrer and qaulity of transferd embryos influenced the pregnancy rates. Similarly, Faizah H.M.S. et. al. (14) revealed that pregnancy rate following non surgical embryo transfer was 62.5% (5 oout 8) in Holstein Friesian dairy cattle . The author indicated that achieving higher pregnancy rates is associated with the interaction of several factors such as; embryos selection, donors and recipients preparation. Another study by Hasler (15) was conducted in two different sites with fresh embryo and pregnancy rates results were 68.3% and 77.1% respectively. Whereas, a study carried out in Netherlands indicated pregnancy rates as 56.1% with using frozen thawed embryos. Pregnancy rates for two studies has been done in the United States were 58.4% and 68.7% respectively (14). It has been reported by many studies that there are several factors which may affect the pregnancy rates after embryo transfer such as environmental factors, heat stress, nutrition system, season, embryo quality and synchronisation procedures. Vasconcelos et al. (3) compared the pregnancy rates following the use of AI and embryo transfer using fresh embryos in Holstein dairy cattle in Brazil, in order to examine the multiple factors which can affect the fertility in lactating dairy cows. The pregnancy rates following embryo transfer to recipients were 58.7% at day 25 and 45.8% at day 46 for embryo transfer, however, the pregnancy rates following AI were 36.5% at day 25 and 33% at day 46. These results indicated that the use of fresh embryos from non-lactating dairy cows which were transferred give better results than using AI. In addition, ovulation failure can be attributed to environmental and stress factors, as a result they may cause infertility in dairy cattle. Besides, embryo transfer with the use of fresh embryos might be used as a tool to maintain high pregnancy rates in lactating dairy cows (3). Additionally, Hasler J.F. (1), pointed out that three main factors can affect embryo transfer such as: donors superovulation, quality of embryos, age of embryo, Embryo handling and freezing.

Bode et al. (16) reported that the protein intake may has the potential to affect early embryo development in lactating dairy cows. He also stated that there are no differences in the quantity or stage of development of embryos which were collected from cows with low plasma urea nitrogen (PUN) or cows with high PUN. Nevertheless, higher pregnancy rate result by the transfer of embryos from cows with low PUN than the embryos from cows with high PUN. Increasing the content of crude protein in the diet that fed to energy-adequate heifers does not affect the response to the superovulation treatment, and the number of embryos recovered, however, it can be beneficial to the quality of embryos (17). Pregnancy rate following embryo transfer in heifer recipients was associated with embryo quality grade, season, and whether embryos were fresh or frozen/thawed. Lactating recipient cows tended to have a lower pregnancy rate after embryos transfer in summer season. Synchronization procedures tends to be associated with pregnancy rate after embryo transfer. Milk production was not correlated with pregnancy rate after embryo transfer. Heat abatement systems are critical to improve embryo production and pregnancy rate after embryo transfer. Synchronization procedures that optimized synchrony of ovulation might increase fertility of recipient cows and eradicate the need for estrous detection (18).

In comparison to MOET program results from other puplished papers. This proramme result categorized as a sucessful study. Besides, our results could have been improved by considering the adequate animals’ requirements in different stages of their life. Besides, there was no effects in terms of the environmental factors, heat stress, and season which could improve the obtaied pregnancy rates. An appropriate management for donors and recipients, adequate nutrition systems and a suitable heat detection procedures can improve MOET programs in the future. Baruselli et al. (19) pointed out that delaying the removal of CIDR (progestogen-releasing devices), combined with the injection of GnRH or pLH 12 hours after the last FSH injection, results in synchronous ovulations, allowing the use of fixed-time AI of donors without the need to estrus detection and without compromising the results. As a consequence the pregnancy rate will be improved in this way. In conclusion, numerous factors could have an influence on embryo transfer in terms of donor and recipient such as preparing donors and recipients, selecting embryo to be transferred into recipients. Obtaining beneficial results depends on recipients pregnancy success, which is important to be at higher rates. Consequently, this will reflect on MOET program in general.

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