Egypt. J. Agric. Res. , 77 (3), 1999.

APPLICATION OF EMBRYO TRANSFER IN FRIESIAN COWS UNDER EGYPTAN CONDITIONS

K.M.E. MOHAMMED AND S.T. ISMAIL

1 Animal Reporduction Research Institute. Agricultural Research Centre Dokki, Giza.
2 Faculty of Veterinary Medicine, Cairo University, Egypt.

(Manuscript received 19 Octobre 1998)

Abstract

Different regimens of superovulation were applied to 33 non-lactating Friesian cows for embryo production. These animals were randomly classifed into 6 groups of superovulatory treatments. Group 1 (n=5) was injected IM with a single dose of 2000 IU pregnant mare serum gonadotropins (PMSG) on day 10-12 of the estrous cycle. Luteolysis was induced by 2 injections of PGF2a (12 h interval) two days after PMSG treatment. The animal that showed signs of estrus was inseminated. Flushing was carried out non-surgically on day 7-9 of the estrus. Group 2 (n=5) and group 3 (n = 7) were treated as group 1, but the dose of PMSG was 2500 IU (Group 2) and 3000 IU (Group 3). The animals of Group 4 (n = 7) were primed with 200 IU/PMSG on day 5 and received 2800 IU/PMSG on day 10 of the cycle. All animals of this group were injected with 2 doses of PGF2a; 48 h after second dose of PMSG. Group 5 (n = 5) and 6 (n = 4) were treated as Group 4, but the priming doses of PMSG were 500 and 1000 IU/PMSG and the main doses of PMSG injected on day 10 were 2500 and 2000 IU/PMSG for Group 5 and 6, respectively. The mean values of CL were 7.6±1.36, 12.8±1.06, 9.57±1.17, 16.0±1.75, 13.8±0.96 and 5.57±0.85, for follicles, it was 1.8±0.66,2.6±0.40, 3.0±0.31, 4.57±0.81, 4.2±0.37 and 2.5±0.29, recovered embryos ova, it was 5.4±1.03, 8.6±0.60, 5.17±0.70, 7.07±1.06, 6.4±0.92 and 2.25 ± 0.85, for fertilized ova, it was 5.0±0.71, 7.0±0.54, 4.67±0.67, 6.5±0.85, 5.0±0.83 and 2.0±0.82, for transferable embryo, it was 4.2±0.79, 6.0±0.71, 3.8±0.54, 5.67±89, 4.4±0.67 and 1.75±0.85, for groups 1,2,3,4,5 and 6, respectively. The best results, as indicated by total ovarian response, CL, recovered embryo/ova, fertilized ova and transferable embryos were obtained in Group 4, when cows primed with 200 IU/PMSG early on Day 5 and 2800 IU/PMSG on Day 10 of the estrous cycle. However, the finding results showed that, regimens of superovulatory treatment had a significant effect on the ovarian response and embryo recovery, and no significant effect on the embryo quality. Calving rates after transfer of single embryo were 44.1% and 42.9% for morula and blastocyst stages, respectively, with no significant differences between the 2 stages, and the overall mean was 43.6%, while, the calving rate after transfer double embryos was 47.6%. The grade of embryos had a significant ($P<0.06$) effect on the calving rate, it was 53.57 and 33.33% for grade I and II embryos, respectively.

INTRODUCTION

Embryo transfer (ET) is a technical science by which fertilized ova are collected from a genetically superior female called donor and transferred for further incubation and development until parturition to surrogate mother of lesser value know as recipient. ET has a great economic potential for rapid improvement of bovine breeds and gives a better chance for genetic improvement and selection (Smith, 1984). Moreover, it provides a safety way of introducing new bloodlines into specific pathogen free herds (Singh, 1987). ET has many advantages, and so, its techniques have now been applied to every species of domestic animals. The chain of events in the process of ET includes, synchronization, superovulation, insemination, embryo collection, evaluation and finally transfer. The availability of embryos for transfer may be increased by inducing superovulation and production of large number of high quality embryos, but the greatest problem with superovulation is the large degree of variation in superovulation response between individuals of the same species. This variation in the response may be contributed to numerous factors, such as, potency, purity, dose and quality of the gonadotrophic preparation, variability in the ovarian follicaular population and its dynamically growth at the time that superovulatory treatment has begun and also to the general health, lactation status of the donor and regimens of the superovulatory treatment (Singh, *et al.*, 1996).

The most apropriate time of gonadotropins administration for superovulation and embryo yield was reported to be at mid-estrous cycle (Days 9-13) in both buffalo (Mohammed, 1991) and cattle (Ware *et al.*, 1987). In fact, this finding is related to the higher number of antral follicles in the ovaries at this period of the cycle (Saumande, *et al.*, 1978) due to the two or three and some times 4 wave patterns of follicular growth through out the estrous cycle (Sirois and Fortune 1988). The first wave in both 2-and 3-waves was identifiable by Day 3 (Day 0 = day of estrus). Many studies in cattle (Petr *et al.*, 1989) have shown that early cycle priming with gonadotropins prior to superovulation increases the subsequent ovulation rate following superovulation. Other authors (MaCmillan *et al.*, 1994) failed to observe any increase in the superovulatory response after similar treatment in cattle.

Production of offspring from ET depends on the viability and development capacity of the embryos. Morphological evaluation of embryos is common and useful in predicting pregnancy rates and can be used at the site of embryo recovery, but this process requiries much practical experience.

The present study was undertaken to evaluate the efficacy of several superovulatory regimens to determine the most appropriate one for producing large number of high quality embryos in Friesian cows in Egypt. Also, the study aimed to evaluate the results of ET in Friesian cows under Egyptian conditions.

MATERIALS AND METHODS

One hudred and nine Friesian cows (33 donors and 76 recipients) were used in this study. The mean age was 3.65±0.71 year, while, the mean parity number and calving-superovulation interval were 1.46±0.09 calving/animal, 362.88±19.98 day, respectively. Estrus cycle of the selected animals was synchronized by a single dose of luteolytic hormone when a well developed and clear CL could be palpated. Double does 11 days apart were used when CL was not clear. After luteolytic hormone injection, the animals were carefully observed twice daily for estrus behavior. Six superovulatory regimens were applied on 6 groups. Group 1 (n = 5), was injected with a single dose of 2000 IU/PMSG on day 10-12 of the estrus cycle. Luteolysis was induced 48 h later by 2 injections of PGF2a (12 hours interval). On the day of estrus, the animals were naturally inseminated by a fertile bull. Flushing was performed non-surgically on Day 7-9 of the estrus (estrus = Day 0) using the method described by Mohammed (1991). Efficacy of superovulation was determined by estimating per rectum the number of ovulation (palpable CL) in each animal and counting the unovulated follicles >10mm, as well as, recovered embryo/ova were counted and classified as fertilized and unfertilized. Further morphological evaluation was done to delineate embryo quality; the evaluation was applied according to Taakeda (1986). Also, recovery percentages of embryo/ova, transferable embryos and fertilization rate were estimeted. Recovered embryos were classified as morula and blastocysts with quality of grade I or II. The animals were categorized as either non-responders (≤2 CL) or responders (≥2 CL). Group 2 (n=5) and Group 3 (2=7) were treated as Group 1, except that the doses of PMSG were 2500 and 3000 IU, respectively. Cows of Groups 4 (n=7), 5 (n=5) and 6 (n=4) were primed on day 5 of the estrus cycle with 200, 500 and 1000 IU/PMSG, respectively, and received other doses of 2800, 2500 and 2000 IU/PMSG on day 10 of the cycle. All animals were administered two doses of luteolytic hormone 48h after second dose of PMSG injection.

Animals used as recipients were synchronized with PG to be in estrus at the same day ±24 h as the donors. Single or double embryos with the same or different qualities and stages were transferred to recipient cows. After transfer, the recip-

Table 1. Ovarian response and embryo recovery inFriesian-cows using different regimens of PMSG (Mean ± SE).

Animal groups (No.)	Ovarian response			No. of** recovered embryo/ova	No. of* fertilized ova	No. of* transferable embryo
	CL**	F*	Total**			
Group 1 (n. = 5)	7.60±1.36^{bc}	1.80±0.66^{b}	9.40±1.93^{c}	5.40±1.03^{ab}	5.00±0.71^{ab}	4.20±0.79^{ab}
Group 2 (n. = 5)	12.8±1.06^{abc}	2.60±0.40^{ab}	15.4±1.40^{abc}	8.60±0.60^{a}	7.00±0.54^{a}	6.00±0.71^{a}
Group 3 (n. = 7)	9.57±1.17^{bc}	3.00±0.31^{ab}	12.57±1.21^{bc}	5.17±0.70^{ab}	4.67±0.67^{ab}	3.83±0.54^{ab}
Group 4 (n. = 7)	16.00±1.75^{a}	4.57±0.81^{a}	20.57±1.90^{a}	7.67±1.06^{a}	6.50±0.85^{a}	5.67±0.89^{a}
Group 5 (n. = 5)	13.80±0.96^{ab}	4.20±0.37^{ab}	18.00±0.95^{ab}	6.40±0.92^{ab}	5.00±0.83^{ab}	4.40±0.67^{ab}
Group 6 (n. = 4)	5.75±0.83^{c}	2.50±0.29^{ab}	8.25±1.03^{c}	2.25±0.85^{b}	2.00±0.82^{b}	1.75±0.85^{b}

CL: Corpus luteum F: Follicles > 10 mm

^{a,b,c} Means in the same column with no common superscripts are significantly different. * ($P<0.05$) ** ($P<0.01$).

Table 2. The influence of superovulatory regimens on the quality of recovered embryos.

Animal groups (No.)	Embryo quality	
	Grade I*	Grade II**
Group 1 (n. = 5)	3.2±0.66^{a}	1.0±0.32^{ab}
Group 2 (n. = 5)	3.6±0.51^{a}	2.4±0.24^{a}
Group 3 (n. = 6)	2.5±0.50^{ab}	1.33±0.33^{ab}
Group 4 (n. = 6)	3.5±0.72^{a}	2.17±0.60^{a}
Group 5 (n. = 5)	3.0±0.45^{ab}	1.4±0.40^{ab}
Group 6 (n. = 4)	1.5±0.65^{b}	0.25±0.2^{b}

* ($P<0.05$) ** ($P<0.01$).

^{a,b,} Means in the same column with no common superscripts are significantly different.

ients were observed for estrus signs, and the success of transfer was estimated when recipients did not return to estrus up to 25 days of transfer, and by rectal palpation 60 days and 4 months after transfer. The data were analyzed using COSTAT compputer package, ver1sion 3.03; copyright 1986 Cottort software.

RESULTS

All cows used in this study were experienced estrus symptoms and responded to superovulatory treatment. However, 2 cows (one in Group 3 and another in Group 4) released pus during flushing due to endometritis which was not diagnosed before superovulatory treatment, therefore, uterine flushing was not completed and the animals were rejected from the collection. The results of superovulation by using different doses and regimens of PMSG have been presented in Tables 1 and 2.

Briefly, the best finding results from different superovulatory regimens, as indicated by total ovarian response (20.57±1.90; $P<0.01$), CL (16.00±1.75; $P<0.01$), recovered embryo/ova (7.67±1.06; $P<0.01$), fertilized ova (6.50±0.85; $P<0.01$)and transferable embryos (5.67±0.89; $P<0.05$) were obtained in Group 4, when cows primed with 200 IU/PMSG early on Day 5 and 2800 IU/PMSG on Day 10 of the estrous cycle. The lower mean values of the previous mentioned parameters were observed in cows of Group 6 injected with 1000 IU/PMSG on Day 5 and 2000 IU/PMSG on Day 10 of the cycle; these values were 8.25±1.03, 5.75±0.83, 2.25±0.85, 2.00±0.82 and 1.75±0.85, respectively.

The highest and lowest means of grade I embrys (3.6±0.51 Vs 1.5±0.65) were observed in animals of Group 4 and 6, respectively, while, the maximum mean of grade II embryos (2.4±0.24) was observed in Group 2 and the minimum mean (0.25±0.2) observed in Group 6 (Table 2). However, the influence of superovulatory regimens on the embryo quality was highly significantly ($P<0.01$) in grade II embryos and grade I embryos ($P<0.05$).

Fifty five recipients cows received single embryo of morula stage (n=34) or blastocyst (n=21). Twenty cows (58.8%) received morulae did not return to estrus up to day 25 of gestation. Forteen cows received blastocyst (66.7%) did not show any signs of estrus up to 25 day of pregnancy. Rectal examination 2 months following transfer revealed pregnancy in 16 cows received morulae (47.1%) and 12 cows received blastocysts (57.1%). These figures were decreased to 44.1% and 47.6% when pregnancy was checked again at 4 months of gestation for recipients received morulae and blastocysts, respectively. At the end of gestation period, 15 from 34

Table 3. Pregnancy rate after transfer of single embryo.

Stage of Embryo	No. Of Recipients	No. of Pregnant Cows (%)			No. of Recipients calving
		25 Day	2 Months	4 Months	
Morula	34	20 (58.8)	16 (47.1)	15 (44.1)	15 (44.1)
Blastocyst	21	14 (66.7)	12 (57.1)	10 (47.6)	9 (42.9)
Total	55	34 (61.8)	28 (50.9)	25 (45.5)	24 (43.6)

Table 4. Pregnancy rate after transfer of double embryos.

Stage of Embryo	No. Of Recipients	No. of Pregnant Cows (%)			No. of Recipients calving (%)
		25 Day	2 Months	4 Months	
Morula	11	7 (63.6)	6 (54.6)	6 (54.6)	6 (54.6)
Blastocyst	5	3 (60)	2 (40)	2 (40)	2 (40)*
Morula & Blastocyst	5	2 (40)	2 (40)	2 (40)	2 (40)
Total	21	12 (57.1)	10 (47.6)	10 (47.6)	10 (47.6)

* One recipient produced single calf and the other produced twin calves.

Table 5. Effect of embryo qualities on the pregnancy rate.

Embryo qualities	No. Of Recipients	No. of Pregnant Cows (%)			No. of Recipients calving (%)
		25 Day	2 Months	4 Months	
Grade I	28	17 (60.71)	15 (53.57)	15 (53.57)	15 (53.57)
Grade II	27	17 (62.96)	13 (48.15)	10 (37.04)	9 (33.33)

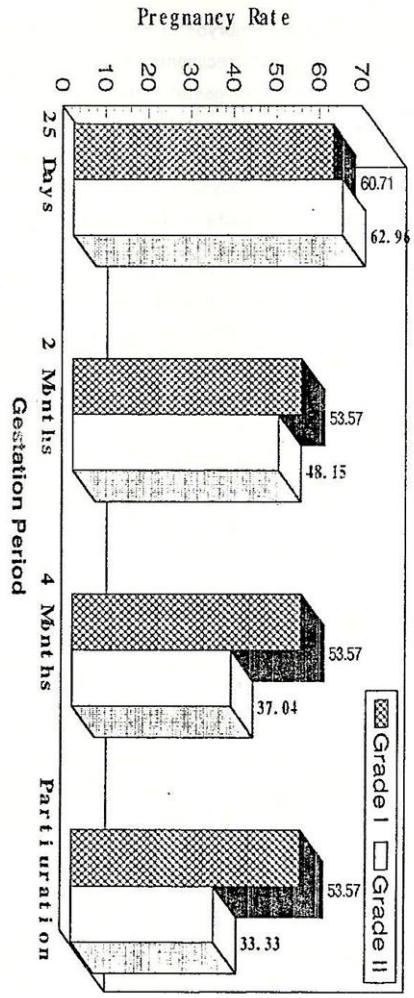

Fig. 1. Effect of embryo qualities on the pregnancy rate.

(44.1%) and 9 from 21(42.9%) recipients which received morulae and blastocysts, respectively, produced calves (Table 3).

Results of transfer double embryos of the same or different stages are presented in Table 4. Twelve out of 21 recipients (57.1%) received double embryos did not return to estrus up to day 25 of gestation (7, 3 and 2 for cows received morulae, blastocysts and blastocyst with morula, respectively). Pregnancy diagnosis 2 months later, showed pregnancy rate 54.6%, 40% for recipients received morulae, blastocysts and morula with blastocyst, respectively. Pregnancy rate was maintained unchanged up to the end of gestation for all recipients.

Twenty-eight recipient cows received 28 embryos (one for each) of grade I, 17 (60.71%) of them did not return to estrus up to 25 days of pregnancy. From these recipients, 15 (53.57%) were checked pregnant 2 months later. There was no decrease in pregnancy rate after day 60 until parturition, and the calving rate was 53.57± (Table 5 and Fig. 1). Ten cows from the recipient females that received grade II embryos (n=27) exhibited a standing estrus up to 18 days following transfer (Day 25 of the transplant estrus cycle). Four out 17 recipient females showed early embryonic death between 25 and 60 days of gestation. However, 3 recipients aborted between 2 and 4 moths of pregnancy and 9 (33.33%) recipients producing offspring of these groups (Table 5 and Fig 1).

DISCUSSION

The Results from the present study showed that, increasing the superovulatory dose of PMSG from 2000 IU (Group 1) to 2500 IU (Group 2) was associated with a significant improvement in the number of ovulation ($P<0.01$), the nubmer of recovered embryos ($P<0.01$), the number of fertilized embryos ($P<0.05$) and the number of transferable embryos ($P<0.05$). In relation to the results obtained in 2000 IU Group (1), the increase in the previously mentioned criteria noted in 2500 IU group was estimated to be 68.4%, 59.3%, 40% and 42.9%, respectively. On the other hand, increasing the superovulatory dose from 2500 IU to 3000 IU (Group 3) adversely affected these criteria. Similarly, Genegenbach, *et al.*, (1978) reported an increase in the ovulation rate in heifers and cows with increasing PMSG dose from 1500 to 2000 and 3000 IU and from 1200 to 2000 IU, respectively. In the same time, Saumande and Chupin (1986) noted a decline in the ovulation rate of heigers from 13.3 CL on application of 2500 IU/PMSG to 8.5 and 2.2 CL when PMSG doses were increased to 5000 IU and 7500 IU, respectively. However, Greve *et al.*,

(1979), in their trial for superovulating 92 cows with 1500-3000 IU/PMSG found that the dose of 3000 IU/PMSG yielded more embryos than 2000 and 2500 IU, but the last 2 doses yielded significantly higher number of viable embryos than did 3000 IU/PMSG. Accordingly, higher doses of PMSG resulted in the formation of large number of follicles. These follicles were developed as a second follicular wave after ovulation due to the long half life of PMSG, and were observed on the ovary at the time of embryo recovery. Such follicles were observed more with high doses of PMSG, and their presence with the corpora lutea would create an unfavourable estrogen/progesterone ratio which caused an allergic effecton embryo transport, and were consequently associated with a low quality of recovered embryos (Dieleman *et al.*, 1987).

As compared to Group 3, injection of small doses of PMSG (200 and 500 IU) early in the cycle significantly ($P<0.01$) improved the ovulation rate by 67.19% (Group 4) and 44.2% (Group 5), respectively. At the same time, the significant increase in the number of recovered embryos, fertilized and transferable embryos in Group (4) was respevtively 48.08%, 38.29% and 50% of those obtained in Group (3). Similarly, Ware *et al.* (1987) reported an increase in the ovulation rate of the FSH primed heifers (17.3 CL) as compared to the control group (5.1 CL). Also, Rajamahendran *et al.* (1987) found a significant increase in the number of CL and the number of recovered embryos obtained in the FSH primed cows. Moreover, Peter *et al.* (1992) reported that, compared to cows treated with a single dose of PMSG, PMSG primed cows had a higher average number of CL (17.8 Vs 7.2) and high percentage of recovered ova (70.2 Vs 60.5), but a lower percentage of fertilized ova (83.8 Vs 91.4); the percentage of good quality embryos did not significantly differ between the two groups (70.7 Vs 79.3). In this aspect, Sirois and Fortune (1988), in their study on dynamics of large follicles ($\geq$5 mm) throughout the estrus cycle in heifers, found that some heifers had 2 waves of follicular growth, the first wave occurred on day 1-5 and the second occurred on days 8-11 from onset of estrus cycle. The mean number of follicles in the second wave was higher than that of the first one. In support, gonadotrophin administration on day 8-10 elicited the highest superovulation response (Putney *et al.*, 1988). Moreover, treatment with small doses of gonadotrophin during the first wave (early in the cycle) was also reported to increase the number of follicles > 4 mm present during the second wave, and the application of a superovulatory hormone at the beginning of the second wave resulted in a better synchronized group of ovulation process. On the contrary to our findings, Lussier and Carruthers (1989) observed a decreased superovulatory response

when gonadotrophins were administered on day 3 of the estrus cycle. The latter authors added that, FSH-P primed heifers had more large follicles (≥7 mm) as detected by ultrasonography at the beginning of superovulation. The mechanism of decreased superovulatory response in gonadotrophin pretreated animals would appear to be associated with reduced endogenous FSH prior to the start of superovulation (Lussier and Carruthers, 1989). These authors reported a positive correlation between the mean FSH concentration for 48 h preceding superovuation and the number of CL per cow. However, discrepancies in the results concerning the pretreatment regimens may have been originated from the variations in the age or breed of cattle used, the different priming regimens, superovulatory used or the time at which the superovulatory treatment had begun. It is interesting to note a significant reduction ($P<0.01$) in fertilized ova when the priming dose of PMSG was increased to 1000 IU. In fact, the high pretreated dose of PMSG may have resulted in enhancing promoting and/or supporting the growth and endocrine activity of one or more large follicles, which would, in turn, release more inhibin (Woodruff *et al.*, 1988), follistatin (Ying *et al.*, 1987) and/or estrogen, thereby suppressing FSH synthesis and/or release consequently, inhibiting growth of small and medium sized follicles (Lussier and Carruthers, 1989). Similarly, Lussier and Carruthers (1989) reported that presence of large active follicles at the start of superovulation may be deterimental to the superovulatory response, and responsible for much of their variability.

The mean values of transferable embryos increased significantly ($P<0.05$) from 4.2±0.79 to 6.00±0.71 with increasing dose of PMSG from 2000 to 2500 IU, and when PMSG dose increased to 3000 IU, the number of transferable embryos decreased ($P<0.05$) to 3.83±0.54. In accordance with results reported by Greve *et al.*, (1979) the dose of 2000-2500 IU/PMSG yielded a significant higher number of viable embryos than did 3000 IU/PMSG. De-Loose *et al.* (1991) cited that induction of superovulation could lead to distrubance in the hormonious development of the preovulatory follicle and its occyte, resulting ultimately to embryos of inferior quality. Moreover, Elsden *et al.* (1978) informed that the uterine environment of superovulated cows found to be harmful for embryos.

In the present study, 55 recipient cows recieved a single embryo (morula or blastocyst), 34 (61.8%) of them did not return to heat 25 days following embryo transfer. Rectal examination at 2 months following transfer confirmed pregnancy in 28 (50.9%) cows. This means that the embryonic loss between day 25 and 60 after embryo transfer was estimated to be 17.7%. This finding was similar to that recorded by Markette *et al.*, (1984) who reported that 19% of the recipients showed

late return to oestrus between day 24 and 48 after embryo transfer. Also, in a study performed by Suzuki *et al.* (1989) the embryonic loss was found to be 17.8% between day 25 and 60. The pregnancy rates indicated that a substantial proportion of developing conceptuses were dying between day 24 and 60, suggesting that these conceptuses were initially able to prevent luteal regression, but subsequently lost this ability. However, early embryonic death which occurred after day 24-26 might be due to premature luteal regression, or it might be due inadequate luteotrophic (LH) or antiluteolytic signal produced by the developing conceptus. The luteotrophic action of LH or LH-like preparations in the cow, as indicated by increased progesterone production or prolongation of the life span of the CL had been clearly demonstrated (Carlson *et al.*, 1971). Other suggestion reported that this death might be due to a change in the nature of the embryonic signal at this time (Christie *et al.*, 1979). Amongst all recipients which received a single fresh morula or blastocyst, 15 (44.1%) and 9 (42.9%) cows, respectively, produced calves at the end of the gestation. Overall mean pregnancy rate was accounted to be 43.6%. This value was higher than that reported in the same species as 31.6 to 38.8% (Subramaniem and Devarajan, 1991), and close to the figures of 43 to 49% reported by Falge et al. (1990). However, higher pregnancy rates were recorded as 75% (Mutiga, 1992). Although the pregnancy rate resulted herein from blastocysts was higher than morulae, this difference was non-significant. This finding was close to the report of Ishimori *et al.* (1993) in which the pregnancy rate resulted from morulae and blatocysts transfer were 38 and 40%, respectively. Also, Kasiraj *et al.* (1993) found that, there was a non-significant difference in the coception rate between buffalo morulae (27%) and blastocysts (20%). However, Niemann (1985) found that, the blastocysts led to a higher pregnancy rate (54.2%) than did the morulae (46.2%).

Out of 21 recipients which received double embryos of the same stage or different stages, 12 recipients (57.1%) did not recturntes trus return to estrus up to 25 days after transfer. Ten recipients (47.6%) were diagnosed pregnant at 2 and 4 months after tranfer until the end of gestation. At the time of parturition, only 11 calves were produced, 9 of them from 9 recipients and only one twin (10%) was produced from one recipient. Our twinning rate (10%) is considered low when compared to other results (23-75%) obtained with bilateral, surgical transfer reported by Suzuki et al (1989). A twinning rate of 47% was obtained by Diskin *et al.*, (1987) when they transferred fresh bovine embryos. Non-surgical and transvaginal embryo transfer procedures yielded lower twinning rates (Boland *et al.*, 1975). The low twinning rate obtained in our study might be due to the difference in quality of

the transferred embryos; in most cases one of low quality (fair or poor), or it might be as a result of a competition between embryos when present in the same horn (ipsilateral to CL) other than bilateral tranfer. This suggestion was confirmed by Rowson *et al.* (1971) who obtained a lower (50%) twinning rate resulting from fresh embryo transfer to the ipsilateral horn than those obtained from bilateral transfer (75). However, pregnancy rates at 4 month after transfer and calving rate were higher in recipients which received double embryos than those which received a ssingle one. It is important to record that the gestation period of the twon pregnancy was 7 days shorter than the mean of single calf pregnancy. Dystocia or difficult delivery was not observed in the cow which has produced the twin, but an assistance was necessary. Also retention of placenta was not observed.

The effect of embryo quality on the embryo survival and pregnancy rate showed that, no significant differences in the survival of transferred embryos between grade I & II embryos at 25 days of gestation; they were 60.71% and 62.96%, respectively. However, the embryonic loss between day 25 and 60 was significantly ($P<0.1$) affected by embryo quality. It was recorded to be 11.76% and 23.53% for grade I and II embryos, respectively. Fifteen (53.57%) of 28 cows received grade I embryos were calved, while, cows received grade II embryos (n. = 27) only 9 (33.33%) recipients produced calves. This meanss that the ability of grade I embryos to establish and maintain pregnancy rate is higher than grade II embryos. Similarly, Brian Mc-Guick (1989) found that, pregnancy rates were 70.4% and 40.7% for grade, I, II and III embryos, respectively. Other author (Donaldson, 1985) recorded that pregnancy rate ranged from 48.9% to 56.1% for superior quality of embryos, and 32.5% to 40.1% for inferior quality. This would indicate that the pregnancy rate decreases in the inferior grade of embryos than superior grade, and it is possible to achieve a high proportion of calving following transfer of high quality embryos.

REFERENCES

1. Boland, M.P., T.F. Crosby and I. Gordon. 1975. Twin pregnacy in cattle established by non surgical egg transfer. Brit. Vet, J., 131:738-780.

2. Brian McGuirk. 1989. The relevance of MOET programmes to developing countries. Theriogenology, 31 (1): 29-39.

3. Carlson, J.C. N. Kazama adn W. Hanse. 1971. Effect of LH on perpherial progesterone concentrations in intact and hysterectomized heifers. Endocrinology, 89, 1530-1533.

4. Christie, W.B. R. Newcomb and L.E. Rowson. 1979. Embryo survival in heifers after transfer of an egg to the uterine horn contralateral to the CL and the effect of treatments with progesterone or HCG on pregnancy rates. J. Repod. fert. 56: 701-706.

5. De-Loose, F.A., M.M. Brevers S.J. Dieleman and A.M. Kruip. 1991. Follicular and oocyte maturation in cows treated for superovulation. Theriogenology, 35: 537-545.

6. Dieleman, S.J., M.M. Bevers and J. Gielen. 1987. Increase of the number of ovulations in PMSG/PG treated cows by administration of monoclonal anti-PMSG shortly after the endogenous LH peak. Theriogenology, 27 (1): 222 (Abstr).

7. Diskin, M.G., T. Mc-Donagh and J.M. Sreenan. 1987. The experimental induction of twin calving in beef cows by embryo transfer. Theriogenology, 27 : 224 (Abstr.).

8. Donaldson, L.E. 1985. Matching of embryo stages and grades with recipient oestrous synchrony in bovine embryo transfer. Vet. Rec., 9.

9. Elsden, R.P., L.D. Nelson and Jr. Seidel. 1978. Superovulating cow with FSH and PMSG. Theriogenology, 9: 17-26.

10. Falge, R., P. Rommel. D. Oesterreich, F. Seifert H. Muller, U. Freymann and B. Draheim. 1990. Cryopreservation of bovine embryos. Arch. Exp. Vet., 44: 127-134 (Abstr.).

11. Gengenbach, D.R., N. Butendieck. P.M., Reik R.L. Scipioni, E.B. Oltenacu and R.H. Foote. 1978. Controlled superovualtion in dairy heifers using PG and PMSG. J. Anim. Sci., 46, 5: 1293-1299.

12. Greve, T., H. Lehn-Jensen and N.A. Rabech. 1979. Practical application of non surgical collection of bovine embyros in Denmark cattle. The Hague, PP. 363.

13. Ishimori, H., K., Saeki, M. Inai. Y. Nagoo, J. Itasaka, Y. Miki, N, Seike and H. Kainuma. 1993. Vitrification of bovine embryos in a mixture of ethylene glycol and DMSO. Theriogenology, 427-433.

14. Kasiraj, R. A.K. Misra, M. MuthaRao, R.S. Jaiswal and N.S. Rangareddi. 1993. Successful culmination of pregnancy and live birth following the transfer of frozen thawed buffalo embryos. Theriogenology, 39 : 1187-1192.

15. Lussier, J.G. and T.D. Carruthers. 1989. Endocrine and superovulatory responses in heifers pretreated with FSH or Bovine follicular folliclar fluid. Theriogenology, 31 (4): 779-794.

16. MaCmillan, K.L., V.K. Taufa and D.L. 1994. Superovulation response to ovine FSH administered during late diestrus to cows fitted with an intravaginal progesterone device. Theriogenology, 41: 234. (Abstr.).

17. Markette, K.L., G.E. Seidel and R.P. Elsden. 1984. Estimation of embryonic loss bovine embryo transfer recipients from progesterone profiles and returns to estrus. Theriogenology, 23: 45-62.

18. Mohammed, K.M. M.E. 1991. Embryo recovery and evaluation in Egyptian buffaloes. Thesis, M.V. Sc. Alex., Univ.

19. Mutiga, E.R. 1992. Use of embryo transfer technology to raise pure bread dairy cows from Zebu cattle. Bull. Anim. Helth. Produc. Africa, 40 (2): 135-136.

20. Niemann, H. 1985. Freezing of bovine embryos. Effect of a one step addition of 1.41 M glycerol. Theriogenology, 23 (2): 369-379.

21. Peter, J. M. Tomanek and F. Jiek. 1992. Relationship between superovulatory response and patterns of pulsatiles secretionof progesterone". Theriogenology, 37: 1301-1310.

22. Petr, J., J. Mika and F. Fllek. 1989. The effect of PMSG priming on the subsequent suerovulatory response in dairy cows. Theriogenology, 33 (5) : 1151-1155.

23. Putney, D.J., W.W. Thatcher., M. Drost, J.M. Wright. and M.A. Delarenzo. Influence of environmental temperature on reproductive performance of bovine embryo donor and recipients in the south west of USA" Theriogenology, 30 (5): 905-922.

24. Rajamahendran, R., R.S. Canseco., C.J. Denbow, F.C. Gwazdauskas and W.E. Vinson. 1987. Effect of low dose of FSH given at the beginning of the estrous cycle and subsequent superovulatory response response in Holstein cows". Theriogenology, 28: 59-65.

25. Rowson, L.E., R.A. Lawson and R.M. Mool. 1971. Production of twins in cattle by egg transfer". J. Reprod. Fert. 25: 261-268.

26. Saumande, J. and D. Chupin. 1986. Induction of superovulation in cycle heifers. The inhibitory effect of large doses of PMSG. theriogenology, 25 (2): 233-247.

27. Singh, E.L. 1987. The disease control potential of embryos. Theriogenology, 27: 9-20.

28. Singh, S.P., P.J., J.S. Hutchinson, R.G. Watt and D.F. Dolman. 1996. Follicular dynamics and superovulatory response in heifers". Anim. Reprod. Sci., 43: 183-190.

29. Sirois, J. and J.E. Fortune. 1988. Ultrasonographic monitoring of ovarian follicular dynamics during the estrous cycle in helfers. Theriogenology, 29: 308 (Abstr.).

30. Smith, C. 1984. Rates of genetic change in farm livestock. Research and development in Agri., 1: 79-85.

31. Subramaniam, A. and K.P. Devarajan. 1991. Zona fracture and its avoidance in cryopreserved bovine ova and embryos". Ind. J. Anim. Sci., 61: 1148-1151.

32. Suzuki, T., Y. Sakai, T. Ishida, S. Matsuda, H. Miura and K. Itoh. 1989. Induction of twinning in cross bred heifers by ipilateral frozen embryo transfer. Theriogenology, 31 (4).

33. Takeda, T. 1986. Identification and evaluation of embryos in bovine embryo transfer". Workshop for veterinarians, Feb., 13-14, Gainsiville, USA.

34. Ware, C.B., D.L. Northry and N.L. First. 1987. Effect of administration of FSH at the beginning of the cycle on the subsequent response to superovulation treatment in heifers". Theriogenology, 27: 292.

35. Woodruff, T.K., J. D'Agostino, N.B. Schwartz, and K.E. Mayo. 1988. Dynamic changes in inhibin messenger RNAs in rat ovarian follicles during the reproductive cycle". Science, 239: 1296-1299.

36. Ying, S.Y., A. Becker, G. Swanson, P. Tan, N. Ling, F. Esch, N.Ueno, S. Schimasaki, and R. Guillemin. 1987. Follistatin specifically inhibits pituitary follicle stimulatory hormone release in vitro". Bioch. Biophs. Ras. Commun., 149: 133-139.

تطبيقات علي نقل الأجنة في أبقار الفريزيان تحت الظروف المصرية

كامل مصطفي السيد محمد و سيد طه إسماعيل

١ محطة بحوث القناطر – مركز البحوث الزراعية – وزارة الزراعة – الدقي - مصر.

٢ معهد بحوث البساتين – مركز البحوث الزراعية – وزارة الزراعة – الدقي - مصر.

تناول البحث دراسة عدة نظم لإحداث التبويض المتعدد لعدد ٣٣ بقرة فريزيان غير حلابة بهدف إنتاج أجنة صالحة للنقل. تم تقسيم هذه الحيوانات إلي ٦ مجموعات. المجموعة الأولي (عددها = ٥ حيوانات) حقنت في العضل ٢٠٠٠ وحدة دولية من هرمون مصل الأفراس الحوامل وبعد الحقن ب ٤٨ ساعة حقنت جرعتان (بينهما ١٢ ساعة) من هرمون البروستاجلاندين وعند ظهور علامات الشياع لقحت الحيوانات. أجريت عملية تجميع للأجنة بالطريقة الغير جراحية في اليوم ٧ – ٩ من التلقيح.

المجموعة الثانية (عددها = ٥ حيوانات) والثالثة (عددها = ٧ حيوانات) تم أعدادها بنفس الطريقة والنظام المستخدم للمجموعة الأولي فيما عدا أن جرعة هرمون مصل الأفراس الحوامل كانت ٢٥٠٠ و ٣٠٠٠ وحدة دولية للمجموعة الثانية والثالثة علي التوالي. المجموعة الرابعة (عددها = ٧ حيوانات) والخامسة (عددها = ٥ حيوانات) والسادسة (عددها = ٤ حيوانات) فقد حقنت ب ٢٠٠ و ٥٠٠ و ١٠٠٠ وحدة دولية من هرمون مصل الأفراس الحوامل في اليوم الخامس من الشباع. وفي اليوم ١٠ – ١٢ من دورة الشياع حقنت الحيوانات ب ٢٨٠٠ و ٢٥٠٠ و ٤٠٠٠ وحدة دولية من هرمون مصل الأفراس الحوامل للمجموعات الرابعة والخامسة والسادسة علي التوالي. وأسفرت النتائج علي أن متوسطات الأجسام الصفراء هي ٧,٦ ± ١,٣٦ و ١٢,٨ ± ١,٠٦ و ٩,٥٧ ± ١,١٧و ١٦,١ ± ١,٧٥ ومتوسطات الأجنة الصالحة للنقل كان ٤,٢ ± ٠,٧٩ و ٦,٠ ± ٠,٧١ و ٣,٨ ± ٠,٥٤ للمجموعات الست علي التوالي . وبالتحليل الأحصائي وجد أن اختلاف نظم التبويض المتعدد له تأثير معنوي علي استجابة المبيض والأجنة المجمعة ولم يكن له تأثير واضح علي حيوية وكفاءة الأجنة. وقد نقلت الأجنة الصالحة للنقل آلي أمهات حاضنة بعد نقل جنين واحد لكل أم هي ٤٤,١٪ للطور التوتي و ٤٢,٩ ٪ للطور البلاستوسيست. أما نسبة الولادة للأمهات التي استقبلت جنينا ذا درجة أولي من الحيوية فكانت ٥٣,٥٧ ٪ و ٣٣,٣٣٪ للأمهات التي استقبلت جنينا ذا درجة ثانية والفرق بين النسبتين معنوي.