PREGNANCY RATE FOLLOWING TRANSFER OF FROZEN BOVINE EMBRYOS ALONG WITH hCG TREATMENT

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ABSTRACT

The present study was an attempt to test the success of undertaking mass transfer of frozen-thawed bovine embryos under village conditions and to evaluate the effect of administration of hCG on pregnancy rate of recipients. A total of 31 Jersey/HF pure bred embryos frozen in 1.4M glycerol were transferred in to synchronized recipients. The recipients were administered two doses of 25mg dinoprost tromethamine at 11 days interval. Following successful induction of estrus, they were divided into 3 groups. Group A (n=12) cows received 2 injections of hCG at the dose rate of 1500 IU per cow on the day of estrus and again at the time of Embryo Transfer (ET). Group B (n=9) cows received a single dose of hCG at the time of ET. Group C (n=10) animals acted as control and received no treatment. In all, 34 embryos were thawed and the cryoprotectant was removed in three steps of decreasing glycerol concentrations. Of the 34 embryos thawed, 3 (8.82%) have undergone degeneration at various stages of cryodilution and were discarded. Transfer of 31 embryos resulted in a pregnancy rate of 69.2% with no significant difference between treatment groups. It may be inferred that frozen-thawed bovine embryos produce satisfactory pregnancy rate following selective transfer into synchronized recipients under field conditions.

Key words: Frozen-thawed embryo, hCG, Glycerol, Pregnancy rate.

INTRODUCTION

In any species the only productive outcome of AI/ ET is the successful establishment of pregnancy, for several factors would contribute to this success. Embryonic mortality was reported as a major cause of spontaneous interruption in gestation (Tefera *et al.*, 2001) and about 30% of embryonic losses occur with in the first 25 days after mating (Lamming *et al.*, 1990). The various factors that might contribute to the death of transferred embryo include: asynchrony between donor and recipient cycles (Hasler *et al.*, 1987); morphologically poor quality embryo (Linder and Wright, 1983); hostile uterine environment (O'Farrel and Hartigan, 1989) and maternal endocrine milieu showing low progesterone levels due to luteal insufficiency (Walton and Stubbings, 1986). It has been demonstrated that administration of hCG in early luteal phase of the cycle has induced ovulation (Fricke *et al.*, 1993) and subsequent formation of CL with resultant increase in plasma progesterone concentrations (Seguin *et al.*, 1997). However, the pregnancy rates may or may not be increased significantly following administration of hCG either after breeding (Tefera *et al.*, 2001) or after embryo transfer (Tribulo *et al.*, 2005). Normally pregnancy rate, following transfer of frozen thawed bovine embryos, was found to be in the range of 20-50% (Leibo,1986). However, occasional high pregnancy rates of 65-70% were also reported in exotic cattle (Spell *et al.*, 2001).

The present work was undertaken to study the pregnancy rate of frozen thawed bovine embryos under field conditions and also to evaluate the effect of hCG administration at the time of estrus and/ embryo transfer on pregnancy rate.

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MATERIALS AND METHODS

Thirty one pure bred embryos frozen in 1.4M (10%) glycerol were transferred in the field into Jersey/HF crossbred cows. The animals screened for selection as potential recipients were distributed in 25-40 Km radius in 5 villages. Healthy, cycling cows with at least 120 days post partum with no history of abnormal calving, repeat breeding or uterine diseases were selected. They were screened and certified negative for brucellosis, Tuberculosis (Tb), Johne's disease (Jd) and Infectious bovine rhino tracheitis - infectious pustular vulvo vaginitis (IBR-IPV) by central veterinary biological research institute, Hyderabad. The general body condition of these animals was improved by deworming and additional supplementation of concentrate ration and green fodder for at least one month before selection. The animals ultimately selected as surrogate mothers were administered two doses of 25mg dinoprost tromethamine (Lutalyse, Upjohn, USA) at 11 days interval and each time the estrus behaviour was closely monitored. They were randomly divided into 3 groups: Group A (n=9) received two i/m injections of hCG (Chorulon, Intervet, Holland) at the dose rate of 1500 IU per cow on day zero (day of estrus) and again on day 7 (day of ET); Group B (n=12) received single injection of hCG 1500 IU on day 7; Group C (n=10) cows received no treatment and acted as control. The ovaries of each animal were examined to confirm the presence of corpus luteum (CL) just before non surgical transfer on day 7

The embryos were frozen in 1.4M (10%) glycerol as per the protocol described by Niemann (1985). Thawing was performed by a brief exposure of straw in air at room temperature and then in a water bath at 37° C for 30 seconds. The embryo from the straw was first removed into 10% glycerol and then immediately transferred to the next step dilutions with decreasing glycerol concentrations viz 6.6%, 3.3% and 0.0% respectively with 0.3M sucrose solution. An additional 4th step dilution was carried out in solution containing 0.15M sucrose in holding medium during the thawing procedure. Both sucrose and glycerol solutions were prepared in PBS supplemented with 0.4% BSA (holding medium). Before transfer, each embryo was washed twice in holding medium and pregnancy in non return cows confirmed by per rectal examination 45-60 days post transfer. Pregnancy rates were analyzed by Chi-square test for comparing percentages (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

Thirty one frozen thawed bovine embryos of various stages and grades were transferred to recipients on day 7 of their synchronized cycle. A high pregnancy rate of 69.2% was obtained with no significant difference between the three groups. It was observed to be 83.33, 55.55 and 60.00% in Groups A, B and C respectively. In agreement with our results, Looney *et al.* (1984) reported no improvement in pregnancy rate following administration of hCG on the day of ET. On the contrary Marques *et al.* (2003) reported significantly higher conception rate in recipients treated with hCG at the time of ET. This variability might be due to different doses of hCG used (Breuel *et al.*, 1990) and also due to variation in the fertility status of recipients.

In the present study, the group that received two injections of hCG on day zero and again on day 7 showed a non significantly higher pregnancy rate over the other two groups of animals. Probably the first injection has caused synchronization of ovulation and the second one produced hypertrophy of existing CL and / or induction of accessory CL there by increasing the progesterone concentrations (Breuel *et al.*, 1990). However, further investigations based on assay of progesterone are needed to support this hypothesis.

The high pregnancy rate obtained in this study may be attributed to (a) intensive selection applied in screening the surrogate mothers coupled with boosting their general body condition one month ahead of actual ET, (b) high rate of embryo survival following thawing and (c) skill and expertise of the operator in performing actual transfer procedure.

During thawing, 70.58% (24/34) embryos retained their pre freezing grade and the quality of 12.90% (4/31) and 9.68% (3/31) of grade I embryos was dipped to grade II and grade III respectively. Only 8.82% (3/34)

embryos have undergone degeneration during thawing. Interestingly the embryonic degeneration in the present study during thawing was much less than the previous reports of up to 20% degeneration during freezing-thawing (Leibo, 1986).

A wide variation in pregnancy rates ranging from 22-55% with embryos frozen in different cryoprotectants and subjected to different methods of cooling and warming rates were reported (Massip *et al.*, 1987). How ever, with improvements in freeze-thaw procedures the pregnancy rates up to 70% have been reported (Tribulo *et al.*, 2005) which were almost equal to the results of the present study.

A non significant increase in pregnancy rate was observed in the present study with blastocysts (71.42%) over morulae (64.71%) stage embryos. Reports on the effect of stage of embryo on pregnancy rate were highly variable and contradictory (Donaldson, 1985). Also pregnancy rate in this study between grades I, II and III embryos was not significantly different presumably due to less number of embryos in each category. This finding is in contrast to the earlier observation that pregnancy rates improved with better embryo quality (Colemann *et al.*, 1987).

From the results of the present study, it may be concluded that frozen embryos of morula and blastocyst stage can be successfully transferred in the field animals with higher pregnancy rates. However, further studies to asses the luteotropic action of hCG at different stages of cycle and, its subsequent effect on embryo survival and successful establishment of pregnancy are warranted.

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