The Indian Journal of Animal Reproduction; 27 (2): 61-64; Dec. 2006

Research Article

A retrospective study on factors influencing pregnancy rate following embryo transfer in cows

MUTHA RAO M[1], UMA MAHESH, Y[2] AND BABU RAO K[3]

Acharya N.G. Ranga Agricultural University, Cattle Project,
LRS, Lam Farm, Guntur - 522 034, (AP)

Received : February 22, 2004
Accepted : November 10, 2006

ABSTRACT

The aim of the present study was to analyze the factors affecting pregnancy rate following non-surgical Embryo Transfer (ET) in cows. Native (Bos indicus) Ongole cows super stimulated during mid luteal phase were subjected to non-surgical embryo collection on day 7 of super estrus and embryos were evaluated based on stage of development and quality. Transfer of 37 embryos resulted in an average pregnancy rate of 56.8 %. It was observed that, irrespective of developmental stage or quality of embryo, pregnancy rates were not compromised when recipients were in estrus ± 24 hrs of donor. The results indicate that transfer grade, site of embryo deposition and quality of recipient have profound influence on conception rate.

Key words: Pregnancy, Ongole, embryo transfer

Pregnancy rate in recipient cows is a direct measure of successful embryo transfer program. Establishing pregnancy through ET involves a complex series of interrelationships between the embryo, its uterine environment and the corpus luteum (Sreenan and Diskin, 1987). Various factors that might influence conception rate following ET in cattle include synchrony between donor and recipient estrus (Rowson *et al.*, 1972; Seidel, 1981; Hasler *et al.*, 1987), embryo quality (Linder and Wright, 1983; Coleman *et al.*, 1987), stage of embryo development (Kunkel and Strichlin, 1978; Hasler *et al.*, 1987), skill of operator and ease of transfer (Gordon, 1975; Schneider *et al.*, 1980), transfer location (Greve, 1981; Wright, 1981; Hasler *et al.*, 1987), site of embryo deposition (Christic *et al.*, 1980; New Comb and Rowson, 1982), maternal endocrine profile (Remsen and Roussel, 1982; Bierschwal and Murphy, 1985; Wilmut *et al.*, 1985) and uterine environment (Walton and stubbings, 1986). In the present study, factors influencing pregnancy rate following non-surgical embryo transfer in native Ongole cows were analyzed retrospectively.

[1]Scientist and corresponding author, LRS, Lam Farm, Guntur – 34, AP. Email: mutharaom@rediffmail.com [2]SRF & [3]Principal Scientist (AR), LRS, Lam Farm, Guntur – 34, AP.

MATERIALS AND METHODS

Parous, lactating, cyclic Ongole breed donors aged 8–10 years were subjected to superovulation during mid luteal phase (day 10–11) of the cycle (estrus = day 0) by administering 200mg of NIH – FSH – P1 (Folltropin – V, Vetrepharm Inc., London, Ontario, Canada) intramuscularly in a twice daily descending dose schedule (40/40, 30/30, 20/20 and 10/10 mg) for 4 consecutive days. Luteolysis was induced by intramuscular administration of 50 mg dinoprost tromethamine (Lutalyse, Upjohn, USA) in 2 equally divided doses at 48 and 60 hrs after initiating superovulatory treatment. The animals were inseminated 3 times from the start of standing estrus and at 12 hrs interval there after using pedigreed frozen semen. Embryos were recovered by non surgical method on day 7 (Newcomb *et al*, 1978). They were evaluated and graded according to the specifications of International Embryo Transfer Society (IETS).

Embryos were non-surgically transferred into native cows and heifers that were within two days of estrous cycle synchrony with the donor. Only those recipients having palpable corpus luteum (CL) were

selected for transfer and the quality was grades as fair (≤ 10mm) and good (> 10mm). All transfers were performed by one person only. Estrus detection was carried out twice daily using vasectomised teaser bull. Pregnancy rates based on day 60 rectal palpation were analyzed by chi-square test for comparing percentages (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

Of the 37 recipients, 21 (56.8%) conceived (Table). A wide range of pregnancy rates between 20 and 75% were reported earlier (Seidel, 1980; Coleman *et al.*, 1987) in exotic cattle indicating influence of several variables.

The pregnancy rates in summer, rainy and winter seasons, respectively were 46.7%, 64.7% and 60.0% with no significant difference between seasons as has been reported by Weaver *et al.* (1986) and Hasler *et al.* (1987). The pregnancy rates were not compromised when the recipients were in estrus as much as 24 hrs before the donor (85.7%) or 24 hrs after the donor (50.0%). However the pregnancy rates were lower (33.0%) when the recipients were in estrus more than 24 hrs after the donor. The results of the present study are similar to earlier observations that asynchrony up to 24 hrs on either side (Sreenan *et al.*, 1975) or even up to 36 hrs before the donor (Hasler *et al.*, 1987) did not lower the pregnancy rate significantly. With this, it is clearly evident now that, in cattle precise estrus synchrony is not as critical as formerly suggested (Lindner and Wright, 1983; Looney *et al.*, 1984).

In the present study, there was no significant difference in pregnancy rates between different stages of embryo as also reported by Shea *et al.* (1976); Schneider *et al.* (1980) and Lindner and Wright (1983). However, others reported that transfer of blastocysts resulted in higher pregnancy rates than that of morulae (Halley *et al.*, 1979; Looney *et al.*, 1980; Wright, 1981 and Donaldson, 1985).

In our study, the pregnancy rate was highest with Grade I embryos (58.8%) followed by Grade II (56.3%) and the lowest was with Grade III embryos (50.0%). But this difference was not significant presumably due to less number of embryos in each category. This is in contrast to earlier reports that pregnancy rates improved significantly with improved embryo quality (Coleman *et al.*, 1987). However, the assignment of a particular grade to an embryo is subjective and the difference in survival rates between close grades of embryos is not always consistent (Sreenan and Diskin, 1987). Sometimes, poor quality embryos produce pregnancy and many morphologically good embryos fail to result in pregnancy suggesting the involvement of various factors in establishing pregnancy (Lindner *et al.*, 1983).

Transfer of embryos into anterior one third of uterine horn resulted in significantly ($x^2 = 7.54$, $P > 0.005$) higher pregnancy rate (70.4%) compared to that of middle one third of horn (20.0%) which is in agreement with earlier reports (New Comb and Rowson, 1980) and the reduced survival of embryos may be due to inadequate signal to the ovary as a result of suboptimal location (New Comb and Rowson, 1980).

The quality of transfer has significantly ($x^2 = 10.09$; $P > 0.005$) influenced the pregnancy rate. The conception rate, in the present study, was found to be higher (71.4%) when embryos were transferred easily and quickly with least manipulation (transfer grade A) than those transfers in which the technician struggled (transfer grade B) during transfer. Similarly Coleman *et al.* (1987) and Thibier and Nibart (1992) reported higher pregnancy rates with transfers performed quickly and smoothly. Probably the skill and experience of the operator determines not only the site of embryo deposition within a selected horn but also the degree of trauma inflicted during transfer (Sreenan and Diskin, 1987).

The embryos were transferred into the horn ispsilateral to the ovary containing CL and the pregnancy rates were not found to be affected by either size of CL or side of transfer. This confirms earlier reports that pregnancy rate in cattle is not affected by either CL quality (Looney *et al.*, 1984 and Donaldson, 1985) or side of transfer (Wright, 1981). In this study, 67.6 % ovulations were observed on right ovary with a significant difference ($x^2 = 9.14$, $P > 0.005$) between the incidence of ovulations on right and left ovaries as also reported by Reece and Turner (1938) and Hasler *et al.* (1980).

The pregnancy rates were significantly ($x^2 = 5.78$,

Table 1: Factors influencing pregnancy rate following embryo transfer in cows.

Attributes		Number of transfers	Number of pregnancies	Pregnancy rate (%)
Total number of transfers		37	21	56.8
Season	Summer	15	7	48.7
	Rainy	17	11	64.7
	Winter	5	3	60
Stage of embryo	Early morula	8	6	75
	Compact morula	17	9	52.9
	Blastocyst	12	6	50
Grade of embryo	I	17	10	58.8
	II	16	9	56.3
	III	4	2	50
Estrus synchrony (hours)*	≤ + 24	10	6	85.7
	Synchronized	14	9	52.9
	≤ -24	10	5	50
	> - 24	3	1	33
Transfer site	Anterior ? of horn	27	19	63.3[a]
	Middle ? of horn	10	2	28.6[b]
Transfer grade (ease of transfer)	A – easy transfer	28	20	66.7[c]
	B – difficult transfer	9	1	14.3[d]
CL status	Fair (≤ 10mm)	28	16	55.2
	Good (> 10mm)	9	5	55.5
Side of CL	Right ovary	25	15	60
	Left ovary	12	6	50
Fertility of recipient	Normal breeder (> 3 AI)	28	19	63.3[e]
	Repeat breeder (> 3 AI)	9	2	28.6[f]
Recipient parity	Heifers	8	5	63.5
	Cows	29	16	55.2

*Recipient came into estrus before (+) or after (-) the donor.
[a-f] figures within a column within each characteristic with different superscripts differ significantly (a-d: P<0.005., e-f: P<0.05).

P > 0.05) lower (28.6%) in repeat breeder cows (inseminated more than 3 times previously) compared to normal breeder cows (63.3%). This is consistent with the hypothesis that the lower fertility in repeat breeder cows may be due to hostile uterine environment causing death of early embryo (O' Farrel and Hartigan, 1989). A non significant difference in pregnancy rates between cows (55.2%) and heifers (62.5%) was observed in this study and these results are in agreement with the reports of Wright (1981).

From the results it may be concluded that pregnancies could be successfully established by transferring embryos to recipients which were in estrus by ± 24 hrs out of phase with donors. The degree of dexterity and site of embryo deposition have significant influence on pregnancy rate in cows.

ACKNOWLEDGEMENT

We thank the authorities of Acharya NG Ranga Agricultural University and the Principal Scientist (AB), Cattle Project, LRS, Lam Farm for their support and physical facilities. Financial assistance from the ICAR under National Agricultural Technology Project – MM Programme on "Production of superior males through embryo transfer technology" is gratefully acknowledged.

REFERENCES

Bierschwal, C.J and Murphy, C.N (1985). EIA progesterone levels in recipient cows on day of transfer – a preliminary report. Proc. Am. Embryo Transfer Assoc pp 37 – 44.

Christie, W.B., New Comb, R and Rowson, L.E.A (1980). Non-surgical transfer of bovine eggs: Investigation of some factors affecting embryo survival. Vet. Rec., **106**: 190 – 193.

Coleman, D.A., Dailey, R.A., Leffel, R.E and Baker, R.D (1987). Estrous synchronisation and establishment of pregnancy in bovine embryo transfer recipients. J. Dairy Sci., **70**: 858 – 866.

Donaldson, L.E (1985). Matching of embryo stages and grades with recipient oestrous synchrony in bovine embryo transfer. Vet. Rec., **117**: 489 – 491.

Gordon, I (1975). Cattle twinning by the egg transfer approach. Seminar on egg transfer in cattle. Cambridge, 10 – 12, December, 1975, 305 – 319.

Greve, T (1981). Bovine egg transplantation in Denmark. Carl Fr. Mortensen. A/s Copenhagen.

Halley, S.M., Rhodes,R.C., Mc Kellar, L.D and Randel, R.D. (1979). Successful superovulation, non-surgical collection and transfer of embryos from Brahman cows. Theriogenology, **12**: 97 – 108.

Hasler, J.F., Bowen, R.A., Nelson, L.D and Seidel, G.E. Jr. (1980). Serum progesterone concentrations in cows receiving embryo transfers. J. Repod. Fert., **58**: 71 – 77.

Hasler, J.F., Mc Cauley, A.D., Lathrop, W.F and Foote, R.H. (1987). Effect of donor – embryo – recipient interactions on pregnancy rate in a large scale bovine embryo transfer program. Theriogenology, **27**: 139 – 168.

Kunkel, R.N and Stricklin (1978). Donor – recipient synchrony, stage of embryo development and post thaw survival of bovine embryos. Theriogenology, **9**: 96.

Lindner, G.M and Wright, R.W. Jr. (1983). Bovine embryo morphology and evaluation. Theriogenology, **20**: 407 – 416.

Looney, C.R., Oden, A.J., Massey, J.M., Johnson, C.A and Godke, R.A (1984). Pregnancy rates following HCG administration at the time of transfer in embryo - recipient cattle. Theriogenology, **21**: 246.

Newcomb, R., Christie, W.B and Rowson, L.E.A (1978). Nonsurgical recovery of bovine embryos. Vet. Rec., **102**: 414- 417.

New Comb, R and Rowson, L.E.A (1980). Investigation of physiological factors affecting non-surgical transfer. Theriogenology, **13**: 41 – 49.

O' Farrel, K.J and Hartigen, P.J (1989). Superovulation and non-surgical egg recovery from normal and repeat breeder dairy cows. Irish Veterinary Journal, January/February, 53 – 55.

Reece, R.P and Turner, C.W (1938). The functional activity of right and left bovine ovary. J. Dairy Sci., **21**: 37 – 39.

Remsen, L.G and Roussel, J.D (1982). Pregnancy rates relating to plasma progesterone levels in recipient heifers at day of transfer. Theriogenology, **33**: 175 – 183.

Rowson, L.E.A., Lawson, R.A.S., Moor, R.M and Baker, A.A (1972). Egg transfer in cow: synchronisation requirements J. Reprod. Fert., **28**: 427 – 431.

Schneider, H.J. Jr.., Castleberry, R.S and Griffin, J.L (1980). Commercial aspects of bovine embryo transfer. Theriogenology, **13**: 73 – 85.

Seidal, G.E. Jr. (1980). Critical review of embryo transfer procedures with cattle. In: Mastroianni, L. Jr. and Biggers, J.D. (Eds). Fertilisation and embryonic development in vitro. Plenum press, PP 323 – 353.

Seidel, G.E. Jr. (1981). Superovulation and embryo transfer in cattle. Science, **211**: 351 – 358.

Shea,B.F., Hines, D.J., Lightfoot, D.E., Ollis, G.W and Olson, S.M (1976). The transfer of bovine embryos. In: Egg transfer in cattle, L.E.A. Rowson (ed.), Commission of the European Communities, Luxemburg pp 145 – 152.

Snedecor, G.W and Cochran W.G (1967). Statistical methods. 6th Ed. Iowa state University press, Ames, Iowa.

Sreenan, J.M and Beehan, D and Mulvehill, P (1975). Egg transfer in the cow: factors affecting pregnancy and twinning rates following bilateral transfers. J. Repro. Fertil., **44**: 77 – 85.

Sreenan, J. M and Diskin, M.G (1987). Factors affecting pregnancy rate following embryo transfer in the cow. Theriogenology **27**: 99 – 113.

Thibier, M and Nibart, M (1992). Clinical aspects of embryo transfer in some domestic animals. Anim. Reprod. Sci., **28**: 139 – 148.

Walton, J.S and Stubbings, R.B (1986). Factors affecting the yield of viable embryos by superovulated Holstein – Friesian cows. Theriogenology, **26**: 167 -177.

Weaver, L.D., Galland, J., Sosnik, U and Cowen, P (1986). Factors affecting embryo transfer success in recipient heifers under field conditions. J. Dairy Sci., **69**: 2711.

Wilmut, I., Sales, D.I and Ashworth, C.J (1985). The influence of variation in embryo stage and maternal hormone profiles on embryo survival in farm animals. Theriogenology, **23**: 107 – 119.

Wright, J.M (1981). Non-surgical embryo transfer in cattle. Embryo-recipient interactions. Theriogenology, **15**: 43 – 56.