PREGNANCY RATES IN VITRIFIED CROSSBRED COW EMBRYOS

D.V. PATEL[1] AND C.P. DEVANAND[2]

Sabarmati Ashram Gaushala, Bidaj Farm, PO-Lali, Dist-Kheda, Gujarat-387 120

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ABSTRACT

The objective of the present work was to introduce Embryo transfer technique at farm to get an insight of the frozen thawed embryos performance post-vitrification in terms of quality of embryos and finally resulting into successful pregnancy. Out of 74 embryos vitrified, 53 embryos were used for transfer in 43 recipients resulting into 10 pregnancies (23.26%). Vitrified embryos yielded comparable pregnancy rates similar to glycerol direct transfer embryos but saving time and cost effective freezing protocol.

Key words: Vitrification, embryos, thawing, pregnancy

With the recent advances in the freezing of embryos, vitrification has been found to be promising technique with the rapid acceptance by veterinary and human embryo freezing programmes. At Sabarmati Ashram Gaushala, Bidaj Farm (SAGB), conventional slow freezing method has been used since the beginning of Embryo Transfer Technology (ETT). Recently, vitrification of embryos has been used extensively worldwide, but there are no reports of calves born through this method in India. At SAGB, transfer of Grade I and fresh embryos yielded an average conception rate of 32.67 per cent, while Grade I and frozen thawed embryos resulted in 14.20 per cent (Patel *et al.,* 2009). Initially, 1.4 M Glycerol was used as a medium for freezing of embryos. Later for direct transfers and field application, 1.5 M Ethylene Glycol (EG) was used without much significant improvement in the pregnancy rates, although EG reduced the time between thawing and transfer of embryos. Again to improve upon pregnancies in direct transfers, 1.4 M Glycerol with side columns having 0.5 Sucrose in the straw was used with the pregnancy rates improving to 23.19 per cent.

With a view to adopt an advanced embryo freezing technique and to observe its applicability in farm and field ETT programmes, the vitrification method was introduced at SAGB. Vitrification is the process of solidification of a solution at low temperatures without ice crystal formation, by extreme elevation in its viscosity using cooling rates of 15,000 to 30,000°C/ min (Rama Raju *et al.,* 2006). This phenomenon requires either rapid cooling rates (Rall, 1987) or the use of concentrated cryoprotectant solutions, which depress ice crystal formation and increase viscosity at low temperatures until the molecules become immobilized and has the properties of solid (Fahy, 1986). To achieve rapid cooling (2500°C/min), the exposure time of embryos to cryoprotectant solutions must be short due to the toxic effects of high concentrations of cryoprotectant. However, if the exposure is too short, the penetration of the cryoprotectant will be inadequate and intracellular ice could form, even in the absence of extracellular ice (Otoi *et al.,* 1998). The way to circumvent the noxious effects of cryoprotectants in the vitrification process could be through the use of high cryoprotectant concentrations for short periods of time (Vajta *et al.,* 1998) or increasing the equilibration period by using lower cryoprotectant concentrations (Papis *et al.,* 2000).

Production of *in-vivo* embryos from indigenous and crossbred bovines was done as per the standard procedures. Donors were superovulated using 200 mg

[1]Consultant, Sabarmati Ashram Gaushala, Bidaj Farm
[2]General Manager, Sabarmati Ashram Gaushala, Bidaj Farm.

/ 400 mg (equally divided constant dose) of Folltropin V (Porcine Follicle Stimulating Hormone, Veterepharm, Canada). Flushing, searching, grading and transfers of embryos was done as per the standard procedures (Misra *et al.*, 1990) and by International Embryo Transfer Society Manual (IETS manual). Embryos were vitrified on cryoloops using readymade vitrification kit (Cook IVF, Australia) procured from Biodynamics, India. The embryos in group of 2-3 were vitrified as per the protocol recommended by manufacturer. After loading the embryos, cryoloop was kept in LN_2 vapour for 30 seconds and then the cryoloop was dipped in to LN_2.

Later, vitrified embryos were thawed using thawing kit (Cook IVF, Australia) as per the protocol recommended by manufacturer. The embryos were finally placed in holding media containing 0.4% BSA and were transferred into the recipients of the farm. Out of total 74 embryos vitrified, 53 embryos were used for transfer in 43 recipients resulting in 10 pregnancies (23.26%). The results obtained are lower as compared to the 44.50 per cent pregnancy rate obtained in field trial of vitrification and one step dilution by van Wagtendonk-de Leeuw *et al.* (1997). As described by Visintin *et al.* (2002), and Zanenga (1993), *Bos taurus* embryos showed homogenous behaviour during the freezing process but *Bos indicus* embryos varied in quality after thawing, resulting into lowered pregnancy rates. This can be one of the reason for comparatively lower prengnacy rates in the study. Due to the limited availability of recipients, repeated use of the same recipients for fresh/frozen ETT programme, might have resulted into lowered pregnancy rates.

Although the conception rates obtained using vitrified embryos have not shown a significant improvement when compared to glycerol direct transfer at the farm yet the technique seems to be promising in terms of saving time for freezing, since conventional freezing takes nearly one and half hours to complete one cycle, and the cost incurred for maintaining sophisticated equipments like the biological freezer in the lab can be avoided.

Thus based on the results obtained in the present study, it is concluded that one can adopt the vitrification technique for ETT programmes with no compromise in the freezing and pregnancy results. Well organized farms where ETT is a part of breeding programmes can take up the use of this technique on large scale.

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