

Karyological profile of nuclear donor skin fibroblast cell lines at different passages

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Bhadawari buffalo breed is known for its high fat content (8–10%) in milk. This breed is now endangered due to large scale crossbreeding with Murrah breed for higher milk yield. For *ex situ* conservation of this breed, its skin fibroblast cell lines have been cryopreserved for posterity (Gupta *et al.* 2004). These cells were used for developing clones of this breed of buffalo using NT method by our group. For production of nuclear transfer derived viable embryos, these cell lines hold the key as they are the only source of genetic material in the form of karyoplast. The increased frequency of chromosomal aberrations was reported in multiple passaging of somatic cells in culture (White *et al.* 1999, Kubota *et al.* 2000, Gupta *et al.* 2002, 2005). The aim of the present study was to evaluate the chromosomal profile of Bhadawari buffalo skin fibroblast cell lines at different passages so that the competent donor cells free from chromosomal anomalies for nuclear transfer and production of viable clones can be produced.

The primary cell lines, 4 each from male and female Bhadawari buffalo donors were subjected to multiple passaging till 24th passage according to the protocol described by Gupta *et al.* (2002). Primary cells as well as cells at 3rd, 6th, 9th, 12th, 18th, 21st and 24th passages were used for chromosomal preparations. The skin fibroblast in monolayer at mid log phase with 50–60% confluency were treated with 0.01% colchicine in 25 cm² flask with 4 ml culture medium. After incubation at 37°C for 75 min, culture medium from the flask was transferred into 15 ml centrifuge tube and cells were harvested with trypsin-EDTA treatment. Chromosomal preparations were made by routine air-drying method and the slides were stained in 2% Giemsa stain. Selected metaphases were micro-photographed under 100X oil immersion microscope.

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The key factors in a nuclear cloning experiment include the donor cell production, evaluation of its cloning competence and its cell cycle stage. The skin fibroblasts can be easily collected, multiplied and cryopreserved as an alternative genetic material for the conservation of endangered genetic resources (Hammond 1998). Kubota *et al.* (2000) held that cryopreservation of ear stem fibroblasts have important implications for tissue banking and preservation of endangered species. To date, the overall cloning efficiency using somatic cells have been low, with the reputed efficiency ranging from 0 to 10% (Kubota *et al.* 2000). The low cloning efficiency may be largely attributed to the incomplete reprogramming of epigenetic signals (Enright *et al.* 2003). Various strategies were used to modify donor cells and the nuclear transfer procedure. Majority of these are focused on donor cells (Kasinathan *et al.* 2001, Enright *et al.* 2003). Kubota *et al.* (2000) reported the effect of passage number of donor cells on outcome of nuclear transfer. The cells of later passages (up to 15) supported the clone development to full term. Thus, the cells of higher passages were responsive to nuclear programming.

Maciera-Coelho (2001) held that each time a cell divides, things are not exactly the same as before. The small changes accumulate that have repercussions on cell structure and functions. The major reason for failure of pregnancies with nuclear transfer reconstructed embryos could be due to problems associated with the chromosomal composition of cell lines used as the nuclear donor (Kubota *et al.* 2001). Use of either a polyploid or aneuploid nucleus as the nuclear donor, would result in a high probability of premature termination of pregnancy (Hare and Singh 1979). Therefore, the monitoring of chromosomal aberrations in cultured somatic cells for development of nuclear donor in cloning should be very important.

In the present study, the chromosomal ploidy remained normal (94.00%) up to 12th passage of Bhadawari buffalo skin fibroblast cell lines when they have already undergone 38 to 46 cumulative population doublings. The incidence of chromosomal aberrations showed increase as the passages increased. It was 17.55 to 30.50% cells at 24th passage. The

Table 1. Frequency of chromosomal aberrations in male and female cell lines

Passage No.	Cell lines							
	Male				Female			
	BBM16	BBM31	BBM32	BBM79	BBF22	BBF33	BBF37	BBF82
P-0	2.56	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P-3	5.55	1.92	3.70	0.00	0.00	2.50	2.85	0.00
P-6	9.52	3.33	5.00	5.55	7.50	8.00	2.32	5.00
P-9	11.11	5.12	5.00	4.70	6.06	7.70	3.44	13.04
P-12	18.42	7.14	5.00	7.14	10.00	10.20	6.89	13.51
P-15	20.40	13.15	7.50	15.62	16.66	10.00	6.89	17.50
P-18	29.41	15.00	10.00	15.00	20.00	12.19	14.28	21.95
P-21	28.57	12.50	13.79	16.66	22.50	25.00	12.50	25.00
P-24	30.55	23.80	30.30	17.50	28.00	28.88	17.50	29.54

chromosomal aberration was mainly of tetraploidy and aneuploidy types. These results are in conformity to the earlier reports (Kubota *et al.* 2000, Yang and Tian 2000) where numerical aberrations were reported in multiple passaged fibroblasts. The primary cells of all the 4 male and 4 female donor animals were having normal chromosome count of 50, XY/50, XX in over 98% of cells scored. The BBM31, BBM32 and BBM79 cell lines from male donors showed normal ploidy levels in more than 93% of their cells up to passage 12 while BBM16 showed normal chromosomal count in 82% cells. Skin fibroblast cell lines from female donor showed the normal karyotype up to passage 12 in 93.5 to 86.5% cells (Table 1). The cells at later passages showed higher frequencies of chromosomal abnormalities in both male and female donor animals.

There were mainly 2 types of chromosomal aberrations in different passage cells. These were polyploidy as well as aneuploidy types, showing loss of one or more chromosome in the metaphase plates. The incidences of chromosomal aberrations increased as the passage number increased; after passage 12 majority of cell lines had chromosomal aberrations in the frequencies ranging from 5 to 30.5% cells from male donor and from 6.85 to 29.4% cells from female donors. Skin fibroblast cells are excellent genetic material for nuclear transfer experiments as the highest success rate was achieved using these cells in production of viable clones in different species. From these results, it can be concluded that skin fibroblast cells up to 9–12 passages can be safely used as karyoplast donors in nuclear transfer for the development of viable clones whereas at later passage though reprogrammability of cells improve in SCNT but one has to compromise with genetic stability.

SUMMARY

The present study was conducted to evaluate the chromosomal profile of Bhadwari buffalo skin fibroblast cell lines at different passages so that the competent donor cells free from chromosomal anomalies for nuclear transfer and production of viable clones can be produced. It was found that skin fibroblast cattle up to 9–12 passages can be safely

used as karyoplast donors in nuclear transfer for the development of viable clones whereas at later passages though reprogrammability of cells improve SCNT yet one has to compromise with genetic stability.

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REFERENCES

- Enright B P, Kubota C, Yang X and Tian X C. 2003. Epigenetic characteristics and development of embryos cloned from donor cells treated by Trichostatin A or 5-aza-2'-deoxycytidine. *Biology of Reproduction* **69**: 896–903.
- Gupta N, Sharma R, Taneja R and Gupta S C. 2002. Effect of multiple passaging on chromosomal profile of adult donor somatic cells. *Proceedings of 10th International Congress of Asian-Australian Association of Animal Production Societies*. New Delhi from 23rd to 27th September 2002.
- Gupta S C, Gupta N, Tian C X and Yang X. 2005. Karyological profile of bovine clones. *Proceedings of International Conference on Animal Production, USA*. Sep 18–24, 2005.
- Hammond K. 1998. Animal genetic resources for the 21st century. *Acta Agricultura Scandinavica* **28**: 11–18.
- Hare W C D and Singh E L. 1979. *Cytogenetics in Animal Reproduction*. p. 96. Common Wealth Agriculture Bureau, London.
- Kasinathan P, Knott J G and Robel J M. 2000. Isolation of bovine fibroblast in G₁-phase of the cell cycle and *in vitro* development of embryos following nuclear transplantation. *Theriogenology* **53**: 223.
- Kubota C, Yamakuchi H, Todoraki M K, Tabra N, Barber N and Yang X. 2000. Six cloned calves produced from adult fibroblast cells after long term culture. *Proceedings of National Academy of Sciences* **97**: 990–95.
- Macieira-Coelho A. 2001. Relevance of *in vitro* studies for aging of the organism: A Review. *Z. Gerontol Geriat* **34**: 429–36.
- White K L, Bunch T O, Mitalipov S and Reed W A. 1999. Establishment of pregnancy after the transfer of nuclear embryos produced from the fusion of argali (*Ovis ammon*) nuclei into domestic sheep (*Ovis aries*) enucleated oocytes. *Cloning* **1**: 47–54.
- Yang X and Tian X C. 2000. Cloning adult animals: What is the genetic age of the clones? *Cloning* **2**: 117–22.