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Human Umbilical Cord Blood Serum, a Better Alternative of Fetal Bovine Serum

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Abstract.- Serum is essential component of cellular growth medium and consists of complex mixture of low and high molecular weight biomolecules required for cellular growth and maintenance. Fetal bovine serum (FBS) is extensively used in cell culture medium and its requirement is on the increase. Several alternatives of FBS in the form of goat, horse and human serum were tested but failed. Chemically defined medium are also in the market but they are very expensive and do not support growth as efficiently. There are also many scientific and ethical problems with the use of FBS so there is a need for an alternative which should have no ethical and scientific problems. Present study was conducted to test cord blood serum (CBS) as an alternative of FBS. HeLa, rMSC, hMSC, HBC-1 and HBC-3 were grown in the presence of 5, 10 and 15% FBS and CBS for 48 h. All cell lines were grown in the presence of 10% FBS and CBS for 0 to 120 h to calculate doubling time. HeLa, rMSC, and hMSC showed better growth and proliferation in the presence of CBS as against HBC-1 and HBC-3 in the presence of FBS. CBS also resulted in better attachment of HeLa and rMSC in short time. Doubling time of HeLa, rMSC and hMSC was reduced in the presence of CBS, while increased in the case of HBC-1and HBC-3. This study proves that CBS can be a better alternative to FBS as it accelerates the proliferation of mesenchymal stem cells as well as HeLa cells and also helps the cells to adhere better CBS, has therefore, a great potential to be used for cell culture, to humanize the products used for cellular therapy and regenerative medicine.

Keywords: HeLa cells, mesenchymal stem cells, fetal bovine serum, cord blood serum, proliferation.

INTRODUCTION

In vitro cell culturing is extensively in use in research work, for the development and testing of medicine and in industry. Use of Fetal Bovine serum (FBS) is an important component of *in vitro* cell, tissue and organ culture growth and maintenance. FBS is a complex mixture of low and high molecular weight biomolecules and mainly consists of vitamins, minerals, hormones, factors (attachment, growth and spreading) and transport proteins etc. (Maurer, 1986; Klein and Dumble, 1993). The estimated annual production of serum was half a million liter in 1995 (Hodgson, 1995) that has increased many today. For one liter of FBS, two bovine fetuses have to be harvested, and hence in order to meet the need of FBS, millions of bovine fetuses have to be harvested annually (Jochems *et al.*, 2002). There is no decrease in the use of FBS but it is on the increase due to increase in number of research center, and its applications for *in vitro* toxicity testing, *in vitro* fertilization and creation of genetically modified organisms *etc.* (Anonymous, 2001). It was a great initiative to replace the animal testing with *in vitro* cell culture methods and now it is time to replace the FBS with chemically defined medium or with serum from some other source.

In addition there are ethical and scientific problems with the use of FBS. The collection procedure raises ethical concerns because it results in suffering of the animal and fetus (Jochems, 1997). There are also several scientific problems regarding use of FBS including batch to batch variation in composition of FBS viral and mycoplasma contamination and presence of prions etc. In view of these, the reproducibility of the results and safety of the products is compromised (Shah, 1999; Wessman and Levings, 1999). Due to these problems, the regulatory authorities usually prohibit or discourage the use of animal sera or its components for use in products for human (Asher,

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1999) because this usually leads to the production of antibodies against the xenoproteins, local inflammation and non-engraftment (Stute *et al.,* 2004).

Several other alternative of FBS were also tested to prove their efficiency *i.e.,* bovine colostrums, combination of sheep's defibrinated plasma with bovine ocular fluid, human AB and goat serum (Paranjape, 2004; Capiaumont *et al.,* 1996; Filipic *et al.,* 2002). The use of goat serum proved to be little success due to its higher toxicity compared with FBS. Later on soybean lipid mixture was also added to goat serum to reduce its toxicity but longer culture with this mixture also resulted in decreased proliferation and increased cell death (Deshpande *et al.,* 2000). There are many chemically defined media (serum free media) with different combination of growth factors available in the market and some of them are specifically defined according to requirement of particular cell line but so far they have proven to be less effective than serum added medium and also they are costly (Battula *et al.,* 2007; Koller *et al.,* 1998).

One of the allogenic sources of serum is umbilical cord blood serum (CBS). CBS is much easier to isolate compared to FBS and it is relatively free of bacterial and viral contamination. Cord blood is isolated immediately after the delivery of the baby. Generally cord blood is incinerated along with cord or it is stored in cord blood banks. There are no strict ethical concerns on the isolation of CBS as this procedure is non invasive and also does not pose any danger to the life of mother or fetus. It was tested on many different cell lines and has proven to be better than FBS (Phadnis *et al.,* 2006; Shetty *et al.,* 2007). Previous studies have proven that CBS is a rich source of soluble factor that provide better culturing environment to growing cells (Bogunia-Kubik *et al.,* 2003; Broxmeyer *et al.,* 1994).

We wanted to know whether FBS and CBS have similar effect on growth and morphology of different cell lines. So in the present study we have tested the effect of FBS and CBS on the growth and morphology of mesenchymal stem cells from rat (rMSC) and human (hMSCs) origin, HeLa cells and two locally developed breast cancer cell lines. In addition we have calculated the doubling time of all these cell lines in the presence of FBS and CBS.

MATERIALS AND METHODS

Isolation and growth of rMSCs

The femur of 7 week old female wistar rat was surgically removed and cleaned of adhering soft tissues, cut aseptically from the edges and bone marrow was flushed out in falcon tube by syringe containing 10 ml DMEM medium (incomplete medium). The marrow cells were disrupted by passing it several times by 16 gauge needle. 1 ml FBS was added to 10 ml bone marrow flush out and then spun at 450 x g for 5 min at room temperature. Supernatant was removed and the cells were re-suspended in complete medium (DMEM containing (2 mM) glutamine, 10% FBS (PAA), 100 U/ml penicillin, and 100 µg/ml streptomycin). Number of cells were counted and $5x10^6$ cells were added to 75 cm^2 Flask (NUNC). After incubating flask under standard culture conditions for 24 h, cells were washed with PBS to remove non adherent cells and complete medium was added.

Isolation and growth of hMSCs

Bone marrow aspirate was collected (2ml) in EDTA vial (venoject) from 26 years old, male healthy donor, diluted 1:1 by adding PBS-EDTA (1X PBS, 2mM EDTA) and carefully loaded over layer of Lympho Separation Medium (MP). Cells were centrifuged at 2500 rpm for 30 min at room temperature. The layer of mononuclear cells was carefully collected in another falcon tube and washed twice with 10 ml of PBS-EDTA solution and spun at 1600 rpm for 10 min. The cells pellet was finally suspended in complete medium. The number of cells were counted and 2 x 10^6 cells were added to T75 Flask (NUNC). The flask was incubated at 37°C for 24 h with 5% CO_2. Medium was changed after every 3 days until culture became confluent.

Isolation and culture of human breast cancer cell line

Breast cancer tissue sample was collected in complete media vial containing higher concentration of antibiotics (10X penicillin and streptomycin) of confirmed breast cancer patient from Jinnah Hospital, Lahore with the consent of the patient. Samples were transferred immediately to lab for

further processing. The tissue pieces were washed with Ca^{2+} and Mg^{2+} free PBS buffer twice to remove the blood cells, cut into small pieces (~1mm) and put in the cell culture dish (Corning), 10 pieces in one cell culture dish. The pieces were allowed to adhere to the surface for 15 min and 10 ml complete medium was added. The dish was incubated at 37°C with 5% CO_2 in humidified environment for 3 days. The medium was changed after 3 days. When cells started migrating out from explants, the tissue pieces were removed by pipette tip and cells were trypsinized by trypsin-EDTA. The number of cells were counted and sub-cultured into 25cm^2 flask. The two cell lines were labeled as HBC-1 and HBC-3.

HeLa cells

HeLa cells were grown in DMEM until 80% confluency. The cells were trypsinized with trypsin-EDTA and counted with hemocytometer.

CBS collection

Cord blood was collected in bags without anticoagulant from Lady Wallingdon Hospital, Lahore. The blood in 50 ml falcon tube was immediately transferred to the lab, and cells were allowed to settle down for 3 h. The serum part of blood was collected carefully and filter sterilized by passing it through 0.2 µm filter and placed at -20°C until further use.

Comparison of effect of FBS and CBS on cell lines

Complete DMEM medium was prepared with 5, 10 and 15% FBS and CBS. HeLa cells (1x 10^5), hMSC, HBC-1 and HBC-3 (2.5x10^4) and rMSCs (3X10^4) were grown in 5, 10 and 15% FBS and CBS in 6 well plate for 48 h. The cells were grown under standard incubation conditions (37°C with 5% CO_2 in humidified environment). Number of cells were counted with hemocytometer after 48 h. Experiments were done in triplicate. Student's `t' test was used for statistical analysis.

Doubling time of cells in presence of FBS and CBS

rMSCs, HeLa cells, HBC-1, HBC-3 and hMSCs at 5 x 10^4 were added in 6 well plate in the presence of 10% FBS and CBS, separately. The number of cells were counted with the help of hemocytometer after 24, 48, 72, 96 and 120 h. The experiment was done in triplicate. Doubling time and growth rate of cells were calculated in the presence of FBS and CBS using doubling time software available online at (http://www.doubling-time.com/compute.php?lang=en).

RESULTS

Isolation of rMSC, hMSC, HBC-I and HBC-3

rMSC, hMSC, HBC-1 and HBC-3 were successfully isolated by the given procedure. rMSC and hMSCs differ in the morphology as rMSCs are more flattened and hMSC are more like spindle shaped while HBC-1 and 3 are more like fibroblasts (Fig. 1). HeLa cells growth was normal and when they were 80% confluent, they were sub-culture by treating with trypsin-EDTA.

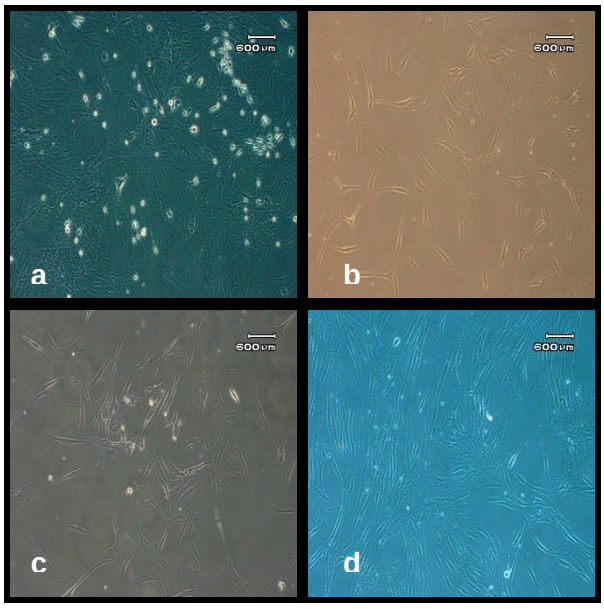

Fig. 1. Normal growth of rMSC (a), hMSC (b), HBC-1 (c) and HBC-3 (d).

Effect of CBS and FBS on HeLa cells

HeLa cells were grown at 1x 10^5 in the presence of 5, 10 and 15% CBS and FBS. The morphology of the cells showed marked change after 24 h of growth (Fig. 2). Cells adopted shape of growing HeLa cells after 24 h of growth but with FBS, they are still round in shape and need more

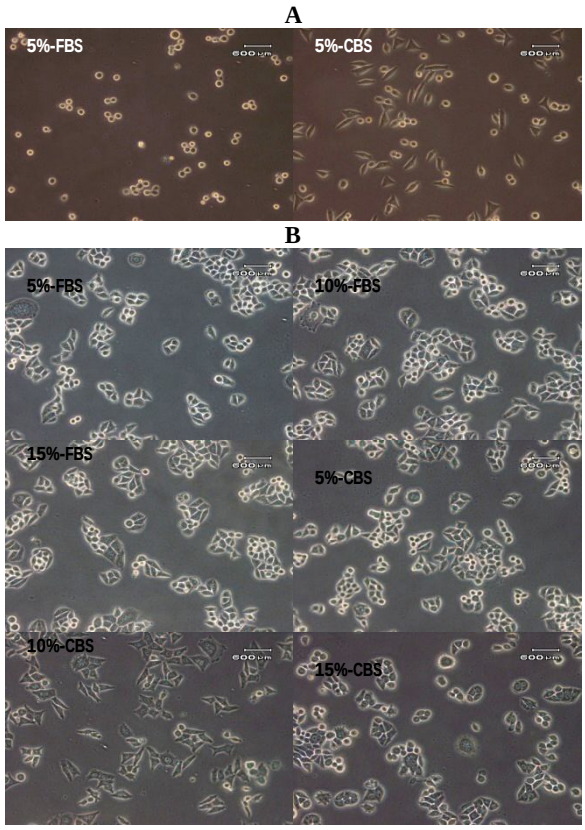

Fig. 2. Growth of HeLa cells in presence of FBS and CBS. A, growth of HeLa cells in the presence of 5% FBS and CBS for 24 h; B, growth of HeLa cells in the presence of 5, 10 and 15% FBS and CBS for 48 h. Top row HeLa cells in the presence of FBS and bottom row HeLa cells in the presence of CBS.

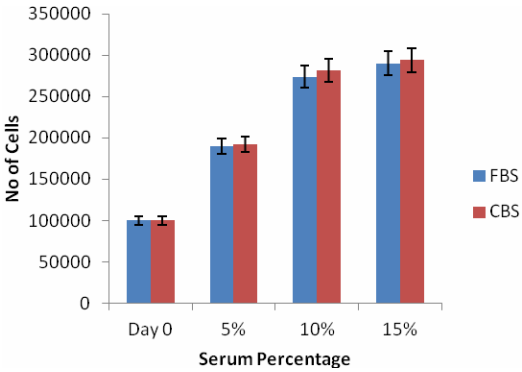

Fig. 3. Growth of HeLa cells in the presence of different concentrations of FBS and CBS. Day 0 are the number of cells added at time 0. The result for 5, 10 and 15% serum concentration are after 48 h of growth.

time to adhere to surface of the flask and adopt its in 5% CBS shape. None of the cells were FBS treatment has the shape of cells with CBS treatment. When cells were grown for 48 h the morphology of the cells was similar but not exactly same. The difference was significant with 10% FBS and CBS after 48 h of growth. There was also increase in number of cells from 5 to 15% FBS and CBS. The increase in number of cells is significant with 10 and 15% of both FBS and CBS as compared to 5% FBS and CBS but there is little advantage in growth of cells with 15% FBS and CBS as compared to 10% FBS and CBS (Fig. 3). Statistically there was no significant difference in growth of HeLa cells in the presence of FBS and CBS.

Effect of CBS and FBS on rMSC

The cells were round in shape in the presence of 5% FBS after 24 h of growth and they became attached after 48 h but instead in the presence of CBS cells were fully attached to plastic surface and their proliferation was much high (Fig. 4A). The growth of cells was much less in the presence of FBS (3.8×10^4 cells in the presence of 15% FBS) compared to CBS (7.6×10^4 cells in the presence of 15% CBS) and there was also change in morphology of the cells. The proliferation rate was much higher in the presence of CBS (Figure 4B). There was significant increase in the number of cells in the presence of CBS as compared to FBS (Fig. 5). Statistically there was no significant difference in the growth of rMSCs in the presence of FBS and CBS.

Effect of CBS and FBS on hMSC

When cells were grown for 24 h in the presence of 5% FBS and CBS, there was only little change in the morphology of cells although there was increase in number of cells in the presence of CBS. After growing of cells for 48 h, there was less change in morphology of cells but proliferation rate increased in the presence of CBS (Fig. 6). There is also increase in the number of cells with the increase in serum concentration in both types of sera. There was decline in number of cells in the presence of 5% serum but cell number increased at 10 and 15% serum concentration. Very little proliferation advantage was observed in the

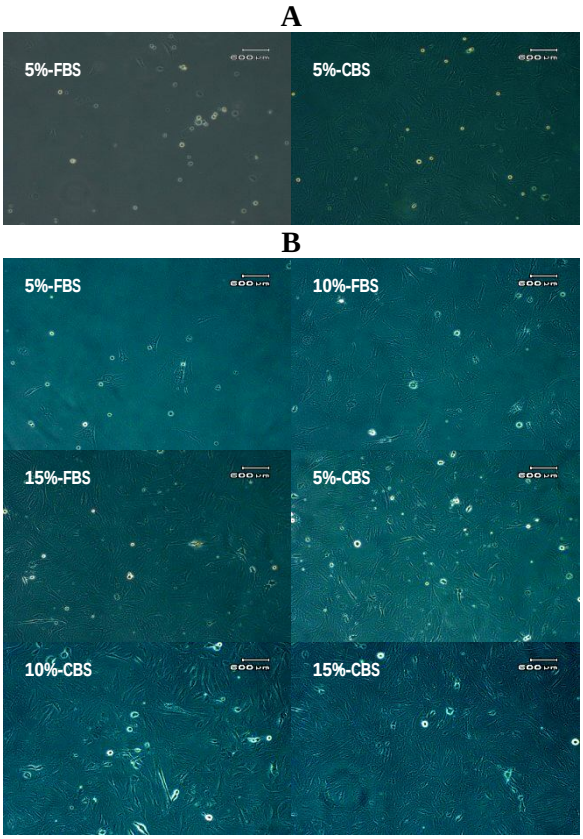

Fig. 4. Growth of rMSCs in presence of FBS and CBS. A, growth of rMSCs in the presence of 5% FBS and CBS for 24 h; B, growth of rMSCs in the presence of 5, 10 and 15% FBS and CBS for 48 h. Top row rMSCs in the presence of FBS and bottom row rMSCs in the presence of CBS.

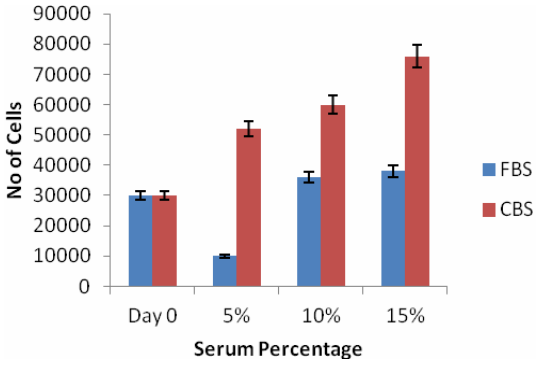

Fig. 5. Growth of rMSCs in the presence of FBS and CBS. There is significant difference in growth of cells in presence of FBS and CBS. Day 0 are the number of cells added at time 0. The result for 5, 10 and 15% serum concentration are after 48 h of growth.

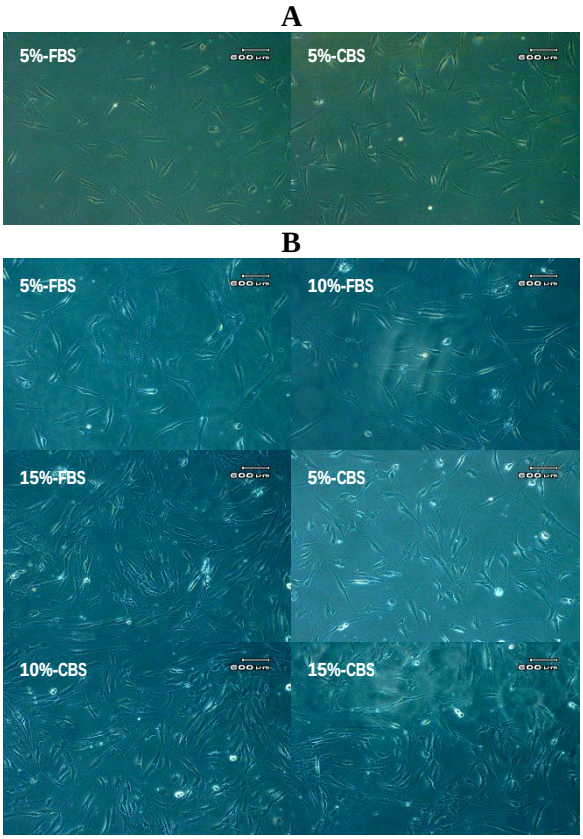

Fig. 6. Growth of hMSCs in presence of FBS and CBS. A, growth of hMSCs in the presence of 5% FBS and CBS for 24 h; B, Growth of hMSCs in the presence of 5, 10 and 15% FBS and CBS for 48 hr. Top row hMSCs in the presence of FBS and bottom row hMSCs in the presence of CBS.

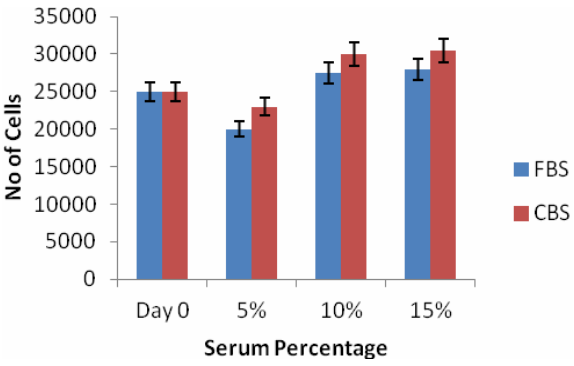

Fig. 7. Growth of hMSC in the presence of 5, 10 and 15% FBS and CBS. Day 0 are the number of cells added at time 0. The result for 5, 10 and 15% serum concentration are after 48 h of growth.

presence of CBS as compared to FBS (Fig. 7). Statistically there was no significant difference in growth of hMSCs in the presence of FBS and CBS.

Effect of CBS and FBS on HBC-I cells

There was change in morphology of cells after 24 h growth in presence of 5% FBS and CBS. The cells were flattened in the presence of FBS but they were more like spindle shaped in presence of CBS (Fig. 8A). Although this difference was resolved after 48 h growth and both FBS and CBS treated cells were appearing spindle shaped. After 48 h growth of cells in the presence of FBS and CBS, there was no apparent change in morphology of cells but as compared to HeLa, rMSC, and hMSC the proliferation rate of HBC-1 was decreased in presence of CBS but cells appeared normal (Fig.8B). There was decrease in the number of cells in 5% FBS and CBS but the number increased in 10 and 15% FBS and CBS. The total number of cells after 48 h of growth in the presence of FBS was more than that in CBS (Fig. 9). Statistically there was no significant difference in growth of HBC-1 in the presence of FBS and CBS.

Effect of CBS and FBS on HBC-3 cells

A significant change in morphology of cells was observed when cells were grown in the presence of 5% FBS and CBS for 24 h (Fig. 10A). When cells were grown for 48 h in the presence of FBS and CBS, there was again significant change in the morphology of cells. The cells became spindle shaped in the presence of FBS but in presence of CBS they became attached to each other and did not appear normal. As it is apparent from Figure 10 there is very little growth of cells in 5% FBS and CBS but growth gradually increased in the presence of 10 and 15% FBS and CBS (Fig. 10B). The number of cells increased in the presence of 5% FBS but it decreased after 48 h of growth. Additionally a significant growth advantage was observed in the presence of FBS compared to CBS (Fig. 11) but the results were totally different in HeLa, rMSC and hMSCs which showed better growth in the presence of CBS. Statistically there was no significant difference in the growth of HBC-3 in the presence of FBS and CBS.

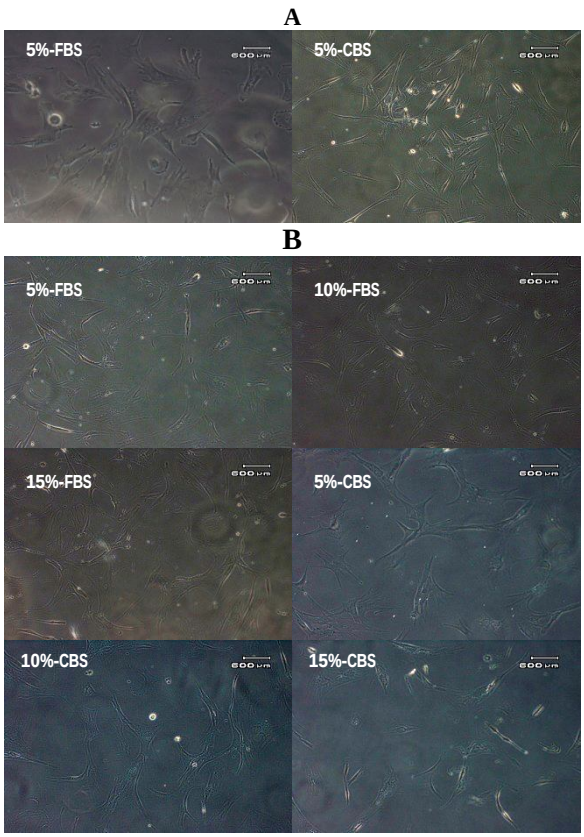

Fig. 8. Growth of HBC-1 in presence of FBS and CBS. A, growth of HBC-1 in the presence of 5% FBS and CBS for 24 h; B, growth of HBC-1 in the presence of 5, 10 and 15% FBS and CBS for 48 h. Top row HBC-1 in the presence of FBS and bottom row HBC-1 in the presence of CBS.

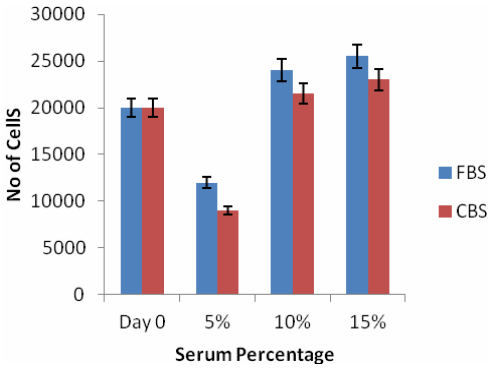

Fig. 9. Growth of HBC-I in the presence of 5, 10 and 15% FBS and CBS. Day 0 are the number of cells added at time 0. The result for 5, 10 and 15% serum concentration are after 48 h of growth.

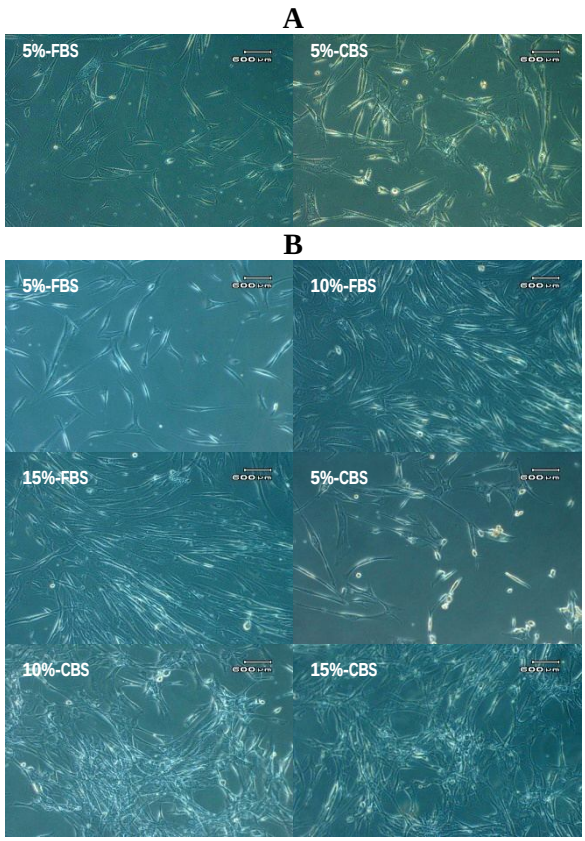

Fig. 10. Growth of HBC-3 in presence of FBS and CBS. A, growth of HBC-3 in the presence of 5% FBS and CBS for 24 h; B, Growth of HBC-3 in the presence of 5, 10 and 15% FBS and CBS for 48 h. Top row HBC-3 in the presence of FBS and bottom row HBC-3 in the presence of CBS.

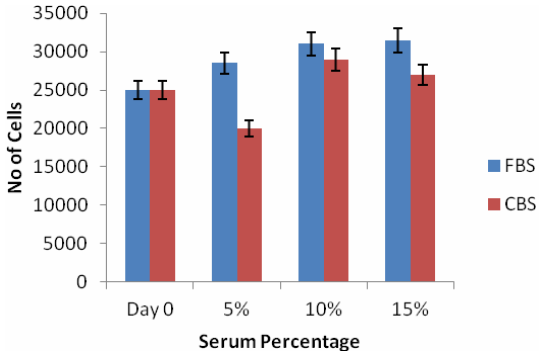

Fig. 11. Growth of HBC-3 in the presence of 5, 10 and 15% FBS and CBS. Day 0 are the number of cells added at time 0. The result for 5, 10 and 15% serum concentration are after 48 h of growth.

Doubling time of cells in presence of FBS and CBS

Doubling time of different cells was different in the presence of FBS and CBS. There was also difference in doubling time among different type of cells as HeLa cells have doubling time 27 h in the presence of CBS and HBC-1 cells as high as 85 h in the presence of CBS. rMSCs, hMSCs and HeLa cells showed rapid growth in the presence of CBS while HBC-1 and 3 showed rapid growth in FBS. Overall doubling time of 3 cell lines was reduced in the presence of CBS, while for 2 cell lives FBS proved to be better growth stimulant. The doubling time of FBS and CBS treated cells in all cell types in this study is significantly different (Table I).

Table I.- Doubling time and growth rate of different cell types in the presence of FBS and CBS.

Cell type	Doubling time (h)		Growth rate*		p value**
	FBS	CBS	FBS	CBS	
rMSCs	47.93	45.34	0.0145	0.0153	0.96
HeLa	28.09	27.31	0.0247	0.0254	.090
HBC-1	78.99	85.01	0.0088	0.0082	0.40
HBC-3	67.65	74.46	0.0102	0.0093	0.69
hMSCs	57.42	55.51	0.0121	0.0125	0.84

*Growth Rate = number of doublings that occur per unit of time
**p value was calculated using t-test

DISCUSSION

Extensive research work is going on all around the world in area of cell biology and growth factors are integral part of growth medium required to grow the cells in *in vitro* conditions. The growth factors are either supplied from FBS, horse serum, goat serum, human adult blood serum or purified growth factors from fetal bovine, goat, horse and human sera. In addition the growth factors are also produced by recombinant DNA technology and added in medium to support the growth of cells. There are some risk factors and shortcomings in using any of these conventional culture medium.

CBS is very easy to isolate after delivery, cheap to process and relatively safe and risk free. It is an ideal source for growth of cells for therapeutic purpose due to lack of xenoproteins. Additionally it is reported to provide ideal system for the growth and proliferation of cells as compared to other sera in use (Broxmeyer *et al.*, 1994; Bogunia-Kubik *et*

al., 2002). Mostly researchers worked on MSCs to test the effect of CBS on growth and morphology of cells and compared it with FBS but in the present study we have studies the effect on HeLa cells, rat and human MSCs and human breast cancer cell lines so could have a better comparison of effect of CBS and FBS on growth and morphology of cells.

Our results reveal that CBS have advantageous effect on growth of HeLa cells after 24 h and cells were fully adherent to plastic surface and morphologically normal, while in presence of FBS cells were still round shaped and did not attach to surface. This is still unclear how CBS stimulated better attachment of cells to plastic surface. Similar results were obtained for the growth of rMSCs after 24 h and mostly cells were attached to surface in the presence of CBS but not in the case of FBS. In case of hMSCs the difference was not as significant as was observed in HeLa and rMSCs and growth and morphology of cells was similar in both of FBS and CBS containing medium. Change in morphology of cells was also observed in HBC-1 and HBC-3 cells in FBS and CBS containing medium after 24 h growth. In this case the number of cells and their attachment was same but their morphology was changes as FBS treated cells were more flattened and CBS treated spindle shaped. So overall, CBS and FBS have effect on morphology of the cells.

The effect of FBS and CBS on proliferation of cells is different in different cell lines. As in some cases CBS promotes the proliferation of cells whereas in others FBS has advantage over CBS. In case of HeLa cells, CBS have advantage over FBS for proliferation of cells but difference is very small. Similar is the case with hMSCs while rMSCs showed much better growth in the presence of CBS and there is significant difference in growth. So in the case of hMSCs and rMSCs CBS provide a better stimulatory environment for growth of these cells. The promotion of growth might be due to release of many growth factors (Insulin like growth factors, transforming growth factor-Beta and vascular endothelial growth factor) by placental cells (Broxmeyer *et al*., 1994) and it is also experimentally proven that CBS activate platelet-derived growth factor and epidermal growth factor signals in MSCs but FBS does not activate these. These results are in accordance with many other findings in which CBS promoted the growth of MSCs (Jung *et al*., 2009). Our doubling time calculation experiment further proves that CBS has proliferation advantage over FBS (Table I) and it reduces the doubling time of cells. CBS gives unique opportunity for short term culture expansion for therapeutic purpose where more number of cells are required in short period of time. It is still unclear as to how CBS promoted growth of rMSC more vigorously than in hMSCs. It probably could be because of efficient attachment of rMSCs to plastic surface in short time compared to hMSCs. There was vast difference in the number of attached cells after 24 h of culture in the presence of CBS and FBS but this was not observed in the presence of FBS case of hMSCs. HBC-1 and HBC-3 showed high proliferation rate and the cells looked normal as against their behaviour in CBS where their number was reduced and the cells had disturbed morphology. CBS promoted the growth of HeLa cells compared to FBS but in the locally isolated two cancer cell lines FBS proved the growth promoter and showed accelerated proliferation rate of breast cancer cells. Further investigation is required to understand the phenomenon. There was another important observation from this study and that was the number of cells and concentration of serum. According to this study there was significant difference in number of cells between 5 and 10% serum concentration but this difference was not significant between 10 and 15% serum concentration. According to present study therefore, 10% serum is optimal for use in the medium and 15% serum will have little advantage on growth of cells.

This study showed that CBS accelerated the proliferation of MSCs and hence can be used to humanize the culture for therapeutic application of MSCs. Additionally CBS helps the cells to quickly attach to plastic surfaces compared to FBS and this might be one of the reasons of accelerated proliferation of cells in the presence of CBS. Almost similar type of results were obtained in the case of HeLa, rMSCs and hMSCs where cells quickly attached to the plastic surface and their number was increased in the presence of CBS. Opposite was however, true for the two breast cancer cell lines where FBS supported the growth of cells and the

cells appeared normal.

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REFERENCES

ANON, 2001. White paper on a strategy for a future chemicals policy. http://europa.eu.int/comm/environment/chemicals/whitepaper.htm. (acces date: 21-08-2003).

ASHER, D.M., 1999. Bovine sera used in the manufacture of biologicals: current concerns and policies of the US. Food and Drug Administration regarding the transmissible spongiform encephalopathies. *Dev. Biol. Stand.,* **99:** 41–44.

BATTULA, V.L., BAREISS, P.M., TREML, S., CONRAD, S., ALBERT, I., HOJAK, S., ABELE, H., SCHEWE, B., JUST, L., SKUTELLA, T. AND BÜHRING, H.J., 2007. Human placenta and bone marrow derived MSC cultured in serum-free, b-FGF-containing medium express cell surface frizzled- 9 and SSEA-4 and give rise to multilineage differentiation. *Differentiation,* **75:** 279–291.

BOGUNIA-KUBIK, K., NATARAJAN, P., BROWN, S., WOLLEY, J., ALCOCER, M., FALLEN, P.R., MADRIGAL, G.A. AND COHEN, S.B., 2003. Cord blood serum affects T cells ability to produce and respond to IL-2. *Cytokine,* **22:** 42–49.

BOGUNIA-KUBIK, K., NATARAJAN, P., MADRIGAL, J.A. AND COHEN, S.B., 2002. The effect of cord blood sera on CD69 expression**.** *Immunol. Lett.,* **84:** 77–80.

BROXMEYER, H.E., BENNINGER, L., YIP-SCHNEIDER, M. AND BRAUN, S.E., 1994. Commentary: a rapid proliferation assay for unknown co-stimulating factors in cord blood plasma possibly involved in enhancement of in vitro expansion and replating capacity of human hematopoietic stem/progenitor cells. *Blood Cells,* **20:** 492-497.

CAPIAUMONT, J., OSTROVIDOV, S., LEGRAND, C., BELLEVILLE, F. AND NABET, P., 1996. Bovine whey: a substitute for FBS in CH0-K1 cell cultures. *In vitro Cell. Dev. Biol. Anim.,* **32:** 8-12.

DESHPANDE, M., KATDARE, M. AND PARAB, P., 2000. Supplementation with soybean lipids reduces goat serum-induced apoptosis in the B-cell hybridoma CC9C10. *In vitro Cell Dev. Biol. Anim.,* **36:** 1-3.

FILIPIC, B., SHEHATA, M., TOTH, S., SCHWARZMEIER, J. AND KOREN, S., 2002. Novel serum replacement based on bovine ocular fluid: a useful tool for cultivation of different animal cells *in vitro. ALTEX: Altern. Anim. Experiment.,* **19:** 15-20.

HODGSON, J., 1995. To treat or not to treat: that is the question for serum. *Biotechnology,* **13:** 333–342.

JOCHEMS, C.E., 1997. *Use, trade and harvest of livestock sera.* http:// www.nca-nl.org/English/Docs/fcreport.doc, 1997 (access date: 21-08-2003).

JOCHEMS, C.E., VAN DER VALK, J.B., STAFLEU, F.R. AND BAUMANS, V., 2002. The use of fetal bovine serum: ethical or scientific problem? *Alternet. Lab. Anim.,* **30:** 219–227.

JUNG, J., MOON, N., AHN, J.Y., OH, E.J., KIM, M., CHO, C.S., SHIN, J.C. AND OH, I.H., 2009. Mesenchymal stromal cells expanded in human allogenic cord blood serum display higher self-renewal and enhanced osteogenic potential. *Stem Cells Dev.,* **18:** 559-571.

KLEIN, R. AND DUMBLE, L.J., 1993. Transmission of Creutzfeldt-Jakob disease by blood transfusion. *Lancet,* **341:** 768.

KOLLER, M.R., MAHER, R.J., MANCHEL, I., OXENDER, M. AND SMITH, A.K., 1998. Alternatives to animal sera for human bone marrow cell expansion: human serum and serum-free media. *J. Hematother.,* 7**:** 413–423.

MAURER, H., 1986. Towards chemically-defined, serum-free media for mamalian cell culture. In: *Animal cell culture: A practical approach* (ed. R. Freshney), IRL Press, Oxford.

PARANJAPE, S., 2004. Goat serum: an alternative to fetal bovine serum in biomedical research. *Indian J. exp. Biol.,* **42:** 26-35.

PHADNIS, S.M, JOGLEKAR, M.V., VENKATESHAN, V., GHASKADBI, S.M., HARDIKAR, A.A. AND BHONDE, R.R., 2006. Human umbilical cord blood serum promotes growth, proliferation, as well as differentiation of human bone marrow-derived progenitor cells. *In vitro Cell. Dev. Biol. Anim.,* **42:** 283–286.

SHAH, G., 1999. Why do we still use serum in the production of biopharmaceuticals? *Dev. Biol. Stand.,* **99**: 17–22.

SHETTY, P., BHARUCHA, K. AND TANAVDE, V., 2007. Human umbilical cord blood serum can replace fetal bovine serum in the culture of mesenchymal stem cells. *Cell Biol. Int.,* **31:** 293–298.

STUTE, N., HOLTZ, K., BUBENHEIM, M., LANGE, C., BLAKE, F. AND ZANDER, A., 2004. Autol ogous serum for isolation and expansion of human mesenchymal stem cells for clinical use. *Exp. Hematol.,* **32:** 1212-1225.

WESSMAN, S.J. AND LEVINGS, R.L., 1999. Benefits and risks due to animal serum used in cell culture production. *Dev. Biol. Stand.,* **99:** 3–8.

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