Characterization of fetal dermal donkey cellular line for equine viruses replication.

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Abstract:
Progress in understanding equine infectious anemia virus (EIAV) replication has been limited by the absence of permissive cell lines. Cells of dermal donkey play multiple roles during the primaries infections of different viruses and serve as reservoirs for viral production. Also these cells have been applied on Chinese donkey leukocyte attenuated equine infectious anemia virus (DLA-EIAV) titration. The aim of this study is to obtain and characterize a cellular line from fetal dermal donkey cells (FDDC) to use for replication equine viruses. With this purpose a primary culture of fetal donkey dermis cells was made and it was sub cultivated until 30 passages. Microscopic observation was carried out to evaluate morphological characteristic of the cultures. Every five passages monolayer confluence time and morphological changes were determinate. Also was measure the grow curve and post freezing survival by cellular counting on Newbauer chamber. The chemical enzymatic disaggregation method was used and a manor confluence time was obtained as a result by each subculture. In a preliminary study EIAV Wyoming strain was inoculated in passage number 30 of FDDC with different percent of monolayer confluence (40% to 90%). After 8 days of incubation morphological changes and monolayer release was observed as evidence of the infective agent presence. In conclusion, this study has identified a useful cell line that could facilitate the study of EIAV expression and replication.

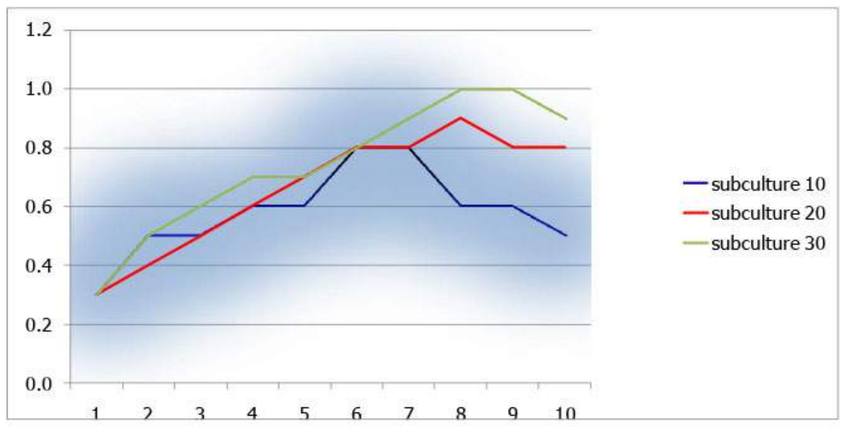
Method

Primary culture of fetal dermal donkey → subculture continuous 10 (Characterization morphological passage 5 at the 10 → Freezing p-10 , and unfreezing . → Realization of the cellular growth curve and post freezing survival → subculture continuous 20 (Characterization morphological passage 11 at the 20 → Freezing p-20 , and unfreezing . → Realization of the cellular growth curve and post freezing survival → Continuous subcultures until p-30 → It proves viral sensibility to herpesviruses and retroviruses (AIE)

Results

Tabla 1. Percent of survival post freezing of the subcultures 10, 20 y 30 FDDC.

% Survival post freezing	%
Subculture 10	59
Subculture 20	61
Subcultures 30	69



Graph 1. Comparison of the growth curve in subcultures of FDDC.

Tabla 2. Characterization Phenotipic for each 5 subcultures of the FDDC.

	Pase 5	Pase 10	Pase 15	Pase 20	Pase 25	Pase 30
Cellular morphology	fibroblástica	fibroblastica	fibroblastica	fibroblastica	Coexistence of cells with morphology fibroblastic and of epithelial type	Fundamentall y Epithelial
Average of time of fork of the monolayer	8 days	6 days	6 days	7 days	5 days	4 days
Characteristic of the monolayer (inhibition for contact, cells in suspension)	I C: si	I C: si	I C: si	I C: si	I C: si	I C: no
	Cells in few suspencion to any solely when defrosting	Cells in few suspencion to any solely when defrosting	Cells in few suspencion to any solely when defrosting	Cells in few suspencion to any solely when defrosting	abundant cells suspencion	Cells Susp. Abundant (turbidity of the means for the abundance of cells)
Requirements of bovine Fetal Serum	10-20%	10-20%	10-20%	10-20%	10%	5%-10%
Range of utilized Split	Max:1:3	Max:1:3	Max:1:3	Max:1:3	Max:1:3	Max:1:5

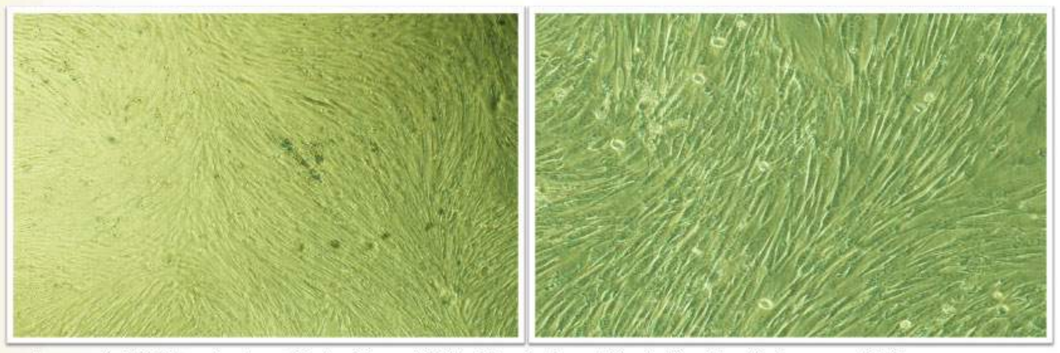


Image 1. FDDC subcuture 20 to 40x and100x. Morphology típicaly fibroblastic in roux of 75 cm.

A line of donkey dermis was obtained starting from a primary cultivation of fetus that had a morphology fibroblasts until the subculture 20 (image 1) and in the subculture 30 happen a spontaneous transformation changing its structure to epithelial (image 2), being evidenced that there is not inhibition by contact with on layers formation.

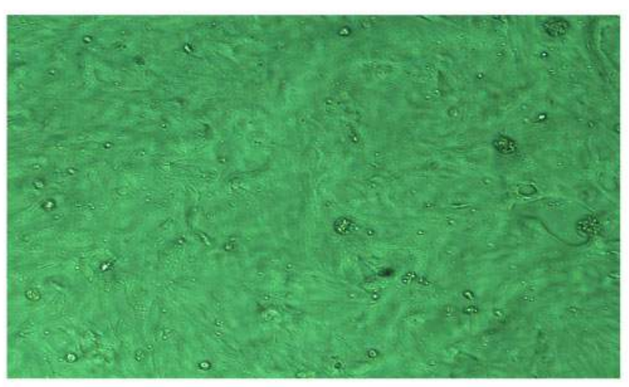


Image 2. FDDC subculture 30 to 100x. Morphology epitelial in roux of 75 cm.

Test of sensibility in retroviruses(EIAV)

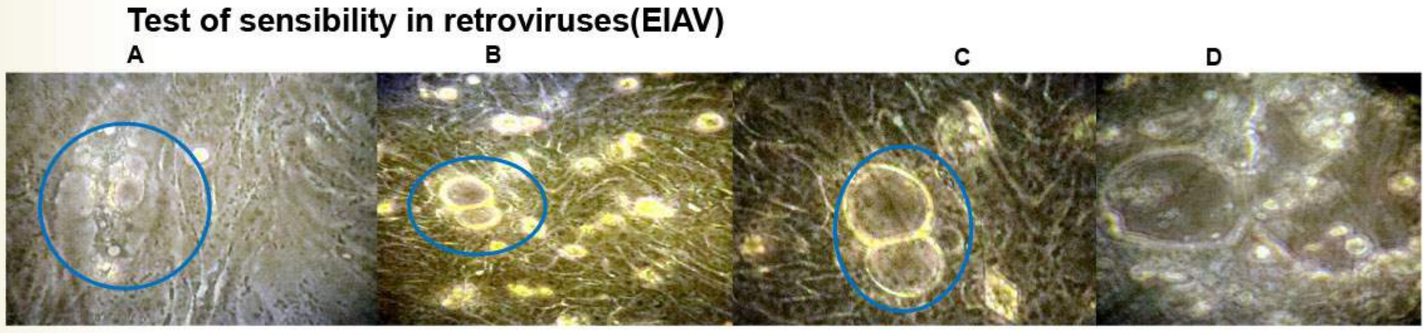


Image 3. Inoculation of a preparation of equine spleen infected to Equine Infectious Anemia Virus (EIAV) During inoculation homogenated equine spleen are observed starting from the third and quarter day the formation of big vacuolas and the cellular destruction. A change the cellular structure to the third day, B - vacuolas formation at 40x in the fourth day, C - image at 60x, D - I begin of the cellular destruction.

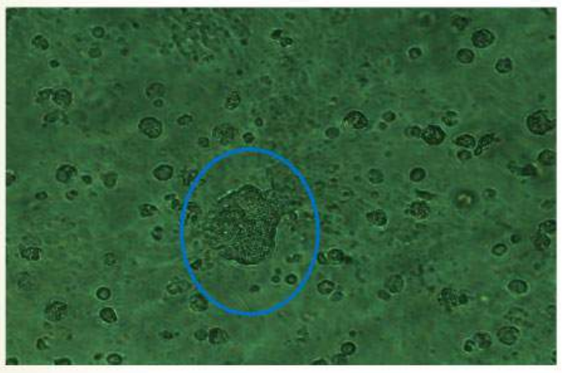


Image 4. Inoculation of a preparation of equine spleen infected to Equine Infectious Anemia Virus (EIAV) wear observed the 5 day the continues of the changes in cellular structure.

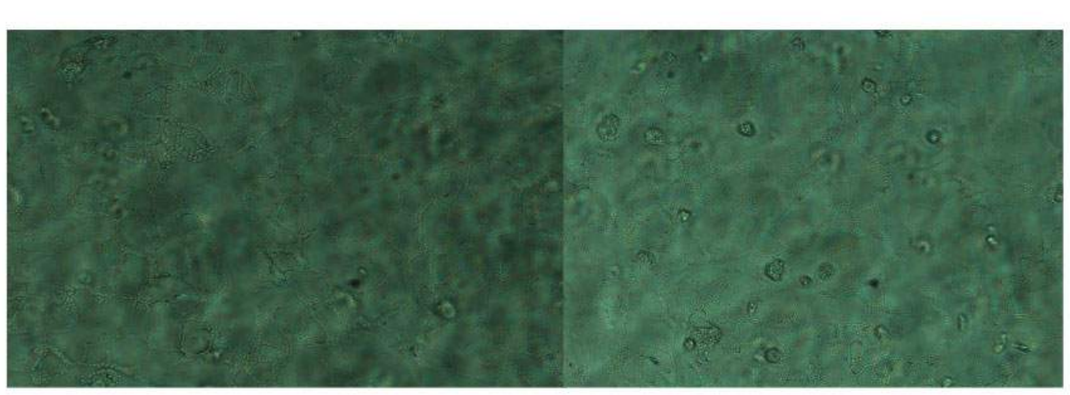


Image 5. . Inoculation of a preparation of equine spleen infected to Equine Infectious Anemia Virus (EIAV) they are observed to the 5 day the monolayer .destruction

Summations

1. Fetal dermal donkey cells obtained was stable from 1 to 27 subcultivo wis fbroblastic morfology
2. An spontaneous morfological transformation from fibroblastic to epitelia was observed in celular line starting from the subculture 27
3. Monolayer modification of thr celular line was observed as evidence of the infetive agent presence to inoculated equine spleen preparation of EIAV Se evidencio modificaciones en la monocapa de la línea celular FDDC) al ser inoculadas con preparado de bazo de un equino enfermo de AIE .

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