

Effect of Centrifugation Time and Speed (G-Force) on Amniocyte Cell Yield

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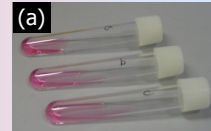
Introduction

- Centrifugation plays an important role in the preparation of cells for cytogenetics therefore it is important to optimise this to achieve maximum cell yield with minimum cell loss.
- We set out to quantify amniotic fluid cells BEFORE and AFTER centrifugation using various centrifugation parameters.

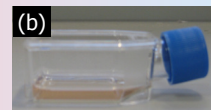
Method

A provisional experiment provided the number of cells representative of a normal harvest. The range used to inoculate the centrifuge tubes was between $0.17 - 0.38 \times 10^6$ cells.

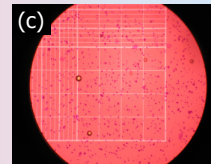
Cultured amniotic fluid (AF) cells from several patients were cultured & pooled into flasks. (a)



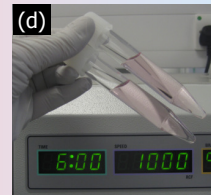
Three to four confluent flasks were trypsinised, pooled then centrifuged; this was enough to inoculate eight centrifuge tubes i.e. a pair of tubes for each centrifuge duration. (b)



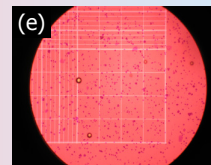
The pellet was resuspended and the cells counted using a haemocytometer. (c)



A known quantity of cells was inoculated into centrifuge tubes containing 5ml Hams F10 without Fetal Bovine Serum (FBS). Each pair centrifuged twice for 4, 6, 8, 10, 12, or 20 minutes for either 1000rpm (179g) or 1500rpm (403g). Supernatant was poured off after the 1st spin and the cells resuspended in 5ml Hams without FBS. After 2nd spin the supernatant was poured off leaving approx 250µl in each tube. (d)



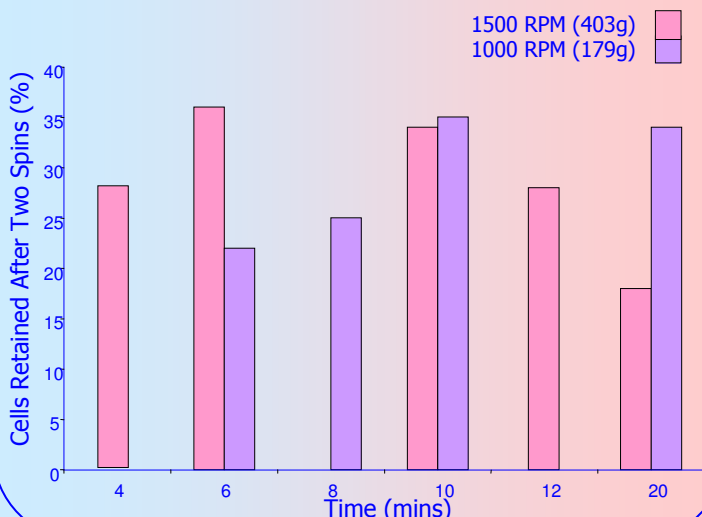
Cells resuspended in supernatant & counted using a haemocytometer. Remaining volume of supernatant was measured and the number of retained cells calculated. (e)



A minimum of 3 pairs of replicate samples were used for each centrifugation time and speed and different batches of pooled cells were used for each set.

Result

Effect of Centrifugation Time and Speed (g) on Amniocyte Cell Yield



Conclusion

Surprisingly low cell yields were seen, up to 85% of cells being lost during two centrifugation steps.

Optimum centrifugation time with the method used was identified as 6 minutes at 403g, with long spin times combined with the higher g-force having a clearly detrimental impact on cell yield.

Things to Consider

- Is the low cell yield due to damaged cells fragmenting and remaining in supernatant?
- Could the yield be increased significantly by pipetting rather than pouring off the supernatant?
- Could the centrifugation process be optimised further? e.g. g-force between 179g and 403g.
- Is there a larger proportion of viable cells at the lower g-force?
- Would the addition of FBS protect the cells more from the effect of centrifugation?
- Would cell yield differ with the various media used during the harvesting process?