Chang Amnio™: A fully supplemented, ready to use medium with an antibiotic optimized for providing analyzable metaphases for amniotic fluid, chorionic villi sampling and tissue cultures

Introduction

A variety of invasive and non-invasive techniques are used for successful prenatal diagnosis to monitor fetal gestation and detect fetal abnormalities. Chromosomes from Amniotic Fluid Cell (AFC), chorionic villi sampling (CVS) and tissues (POC) cultured *in vitro* have been used for more than 40 years to diagnose prenatal genetic abnormalities. A significant advance was reported by Chang et al. (PNAS, 1982) which subsequently led to the development of CHANG MEDIUM®, specifically designed for the culture of prenatal cells and tissues. Both the methods and the nutrient media used in the culture of these cells have evolved considerably over the years.

To achieve optimum efficiency for diagnosis, a culture medium needs to promote rapid cell growth for a large number of colonies, fewer days to harvest, deliver a high mitotic index with sufficient metaphase cells and good chromosome morphology.

When starting the process of creating a new prenatal medium, both AmnioMax™ C-100 and AmnioMax™ II were considered for use as the "control" medium. When run in parallel studies, the AmnioMax™ C-100 outperformed the AmnioMax™ II and was thus chosen as the "control".

Below are two studies that compared the performance of a new, ready-to-use prenatal medium (Chang Amnio™) versus the chosen control medium (AmnioMax™ C-100), both intended for the growth of amniocytes for diagnosis of prenatal abnormalities.

Study One (AFC in situ proliferation assay)

Pooled low passage human AFC cells (from primary AFC aspirates obtained at 15-21 weeks of gestation) were used for cell growth studies. Cells were seeded at $1X10^4$ cells/well in triplicate in a 6-well dish and grown without medium renewal for 6 days at 37°C, 5%CO_2.

Results

The growth performance of Chang Amnio™ was superior for AFC compared to AmnioMax™C-100 and AmnioMax™ II.

(Figure 1).

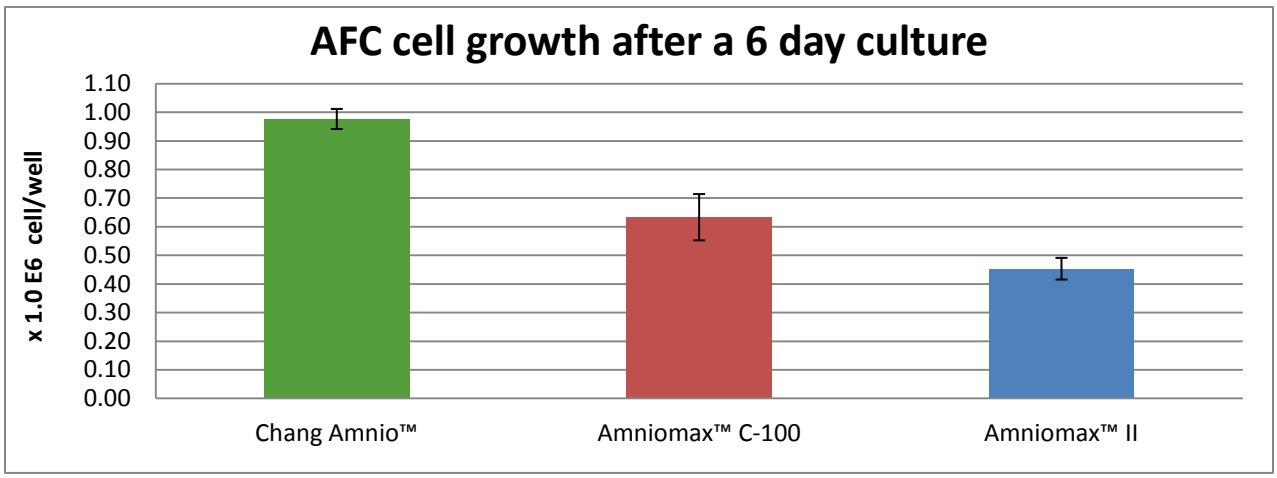

Figure 1: AFC growth after 6 day culture in Chang Amnio™ and AmnioMax™ (initial inoculation 1.23xE04/well). The results are the average of 3 separate experiments ± SEM.

Study Two (Clinical evaluation)

The clinical evaluation of Chang Amnio™ was carried out by an independent clinical Laboratory (Colorado Genetics Laboratory) using their standard amniotic fluid cell protocol. Human amniotic fluid cell (AFC) samples from sixty (60) patients of gestational age between 15 and 21 weeks were used in parallel studies of medium performance. Each patient sample was split into two tubes and centrifuged at 800-1000 rpm for 10 minutes in a clinical centrifuge. The supernatants were aspirated, and the two cell pellets were resuspended with 1 mL of medium per tube. In each case, one tube was resuspended with Chang Amnio™ and the other tube was resuspended with AmnioMAX™ C-100 (Gibco). A total of four 22 mm^2 sterile coverslips were seeded with 0.5 mL of cell suspension each (two coverslips for each type of medium). Each pair of coverslips was maintained in a 35 mm^2 petri dish in a 37°C incubator at 100% relative humidity and 5% CO_2. After approximately 24 hours, 1 mL of the corresponding medium was added to each petri dish. After 3-4 days culture, the medium was changed each 2-3 days until harvest. On the day of harvest, Colcemid® was added for 35 minutes prior to initiating the standard cell harvest and staining procedures.

The clinical evaluations of the coverslips included 3 parameters that the clinical cytogenetics laboratory judged to be most useful in a evaluation of the quality of a culture medium for cytogenetic analysis, namely:

1. Days to Harvest
2. The total number of colonies containing at least 50 cells per colony
3. The number of mitotic figures (metaphase spreads) per coverslip which meet the criteria for analysis

Results

The number of days to harvest varied between 6-8 days, depending on the quality of the specimen. The mean number of days to harvest was approximately 8 for each of the two of media, with no significant difference between them. Chang Amnio™ was comparable to AmnioMax™ C-100 for the average colonies per coverslip and mitotic figures per coverslip.

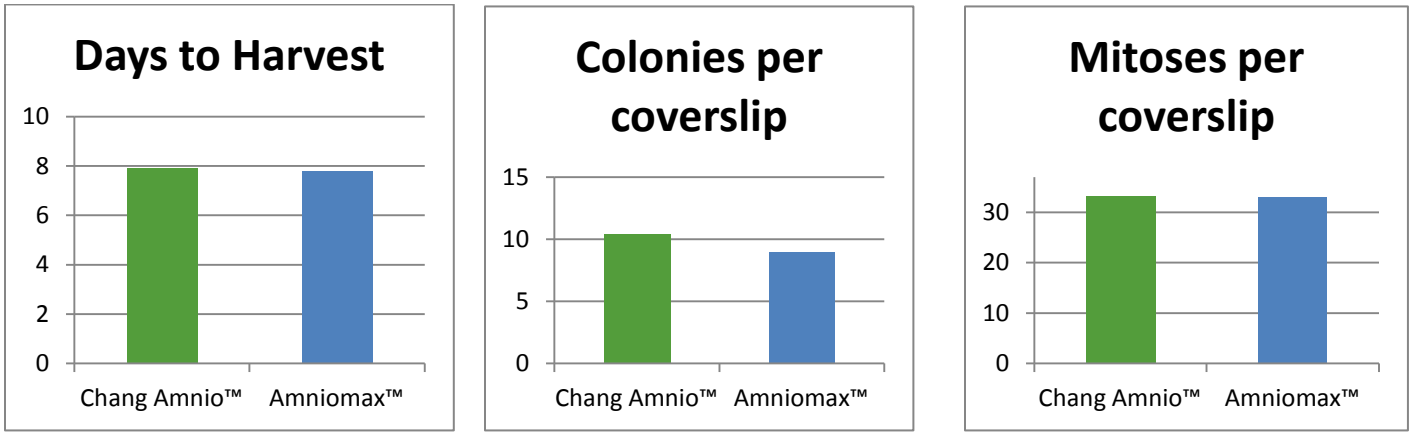

Figure 2: Performance of Chang Amnio™: Clinical analysis of primary AFC samples from 60 patients were evaluated in parallel in 3 different lots of Chang Amnio™ compared to AmnioMax™ C-100. The results reported here are the mean value for n=60.

Conclusion

Chang Amnio™ is a new, high performing complete medium designed and optimized for providing analyzable metaphases for amniotic fluid, chorionic villi sampling and tissue (POC) cultures**.** It provides a reliable and consistent number of mitotic cells suitable for karyotyping for more accurate cytogenetic analysis. Chang Amnio™ is an excellent medium to choose when culturing prenatal cells.