HDF Cell Constraints: Viability and Proliferation Experiments

Experimental Objectives

- To assess HDF cell viability under conditions of ethanol toxicity
- To analyze the effects of 1%, 5%, and 10% FBS on:
  - Percentage of cell nuclei entering S-phase at day 2
  - Cell count at days 2, 5, and 7
  - Cell doubling rate over 7 days

Live/Dead Assay Evaluates Cell Viability

- Nine wells of HDF cells were seeded and incubated for two days
- Established three wells per condition:
  - 250 µL PBS, 100 µL Live/Dead dye
  - 250 µL ethanol, 100 µL Live/Dead dye
  - 250 µL PBS, 2 drops ethanol, 100 µL Live/Dead dye
- Cell viability visualized via fluorescent microscope
  - Calcein AM stained viable cells green
  - Ethidium homodimer stained dead cell nuclei red

PCNA Assay Evaluates S-Phase Nuclei

- HDF cells were seeded in five wells:
  - Three 10% FBS control wells
  - One well per 1%, 5%, and 10% FBS conditions
- Cells were stained with Anti-PCNA and hematoxylin
- Assay was visualized via light microscopy
  - Anti-PCNA stained S-phase nuclei red
  - Hematoxylin stained cell nuclei blue

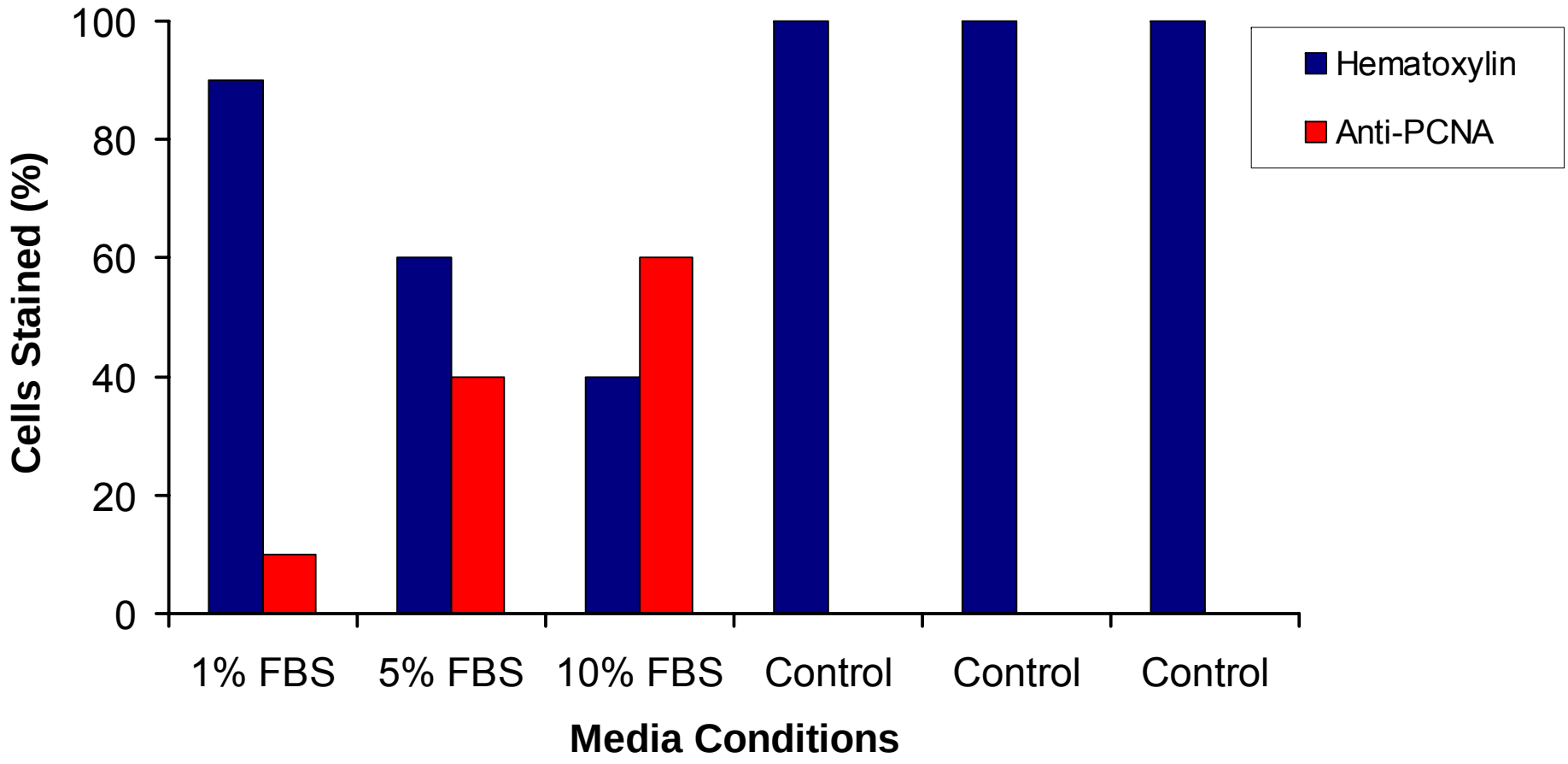
Measuring Proliferation with 1%, 5%, and 10% FBS

- HDF cells were seeded for time points:
  - Day 0: Six control wells with 1% FBS
  - Days 2, 5, and 7: Three wells each with 1%, 5%, and 10% FBS
- At each time point:
  - Visualized cell density via light microscope
  - Trypsinized cells and lifted for counting with a coulter counter

Ethanol Exposure Decreases Cell Viability

Well Condition	Calcein AM Stained (Green-Alive)	Ethidium Homodimer Stained (Red-Dead)
250 μL PBS	90-100% of cells in all three wells	<10% in all three wells
250 μL Ethanol	No stained cells	100% in all three wells
250 μL PBS, 2 drops Ethanol	<10% of cells in center of all three wells 90% of cells on edges of all three wells	90-100% of cells in center of all three wells 10% of cells on edges of all three wells

FBS Decrease Reduces Anti-PCNA Staining

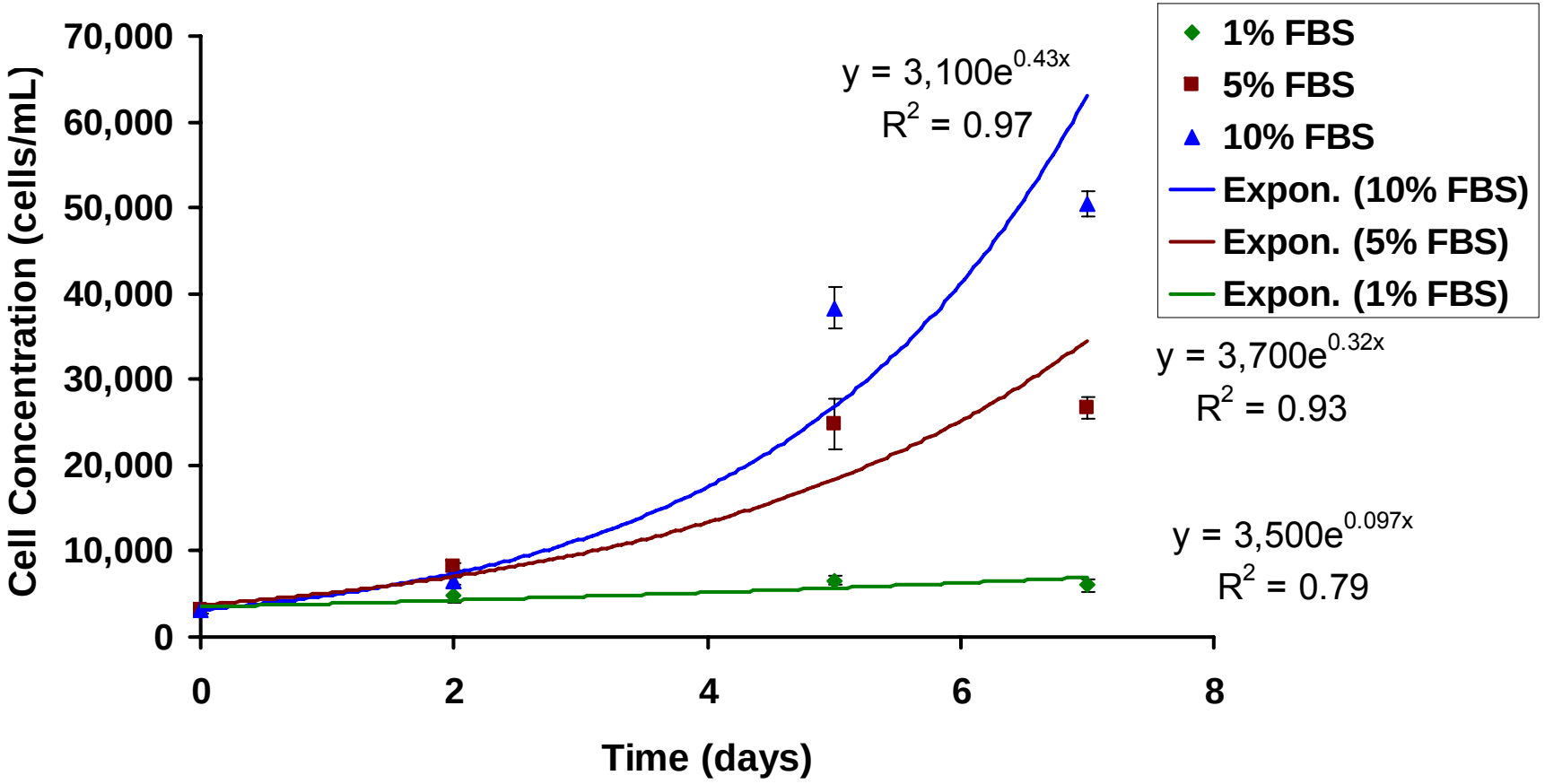


Data Provided by: XXX

S-Phase Nuclei Percentage Drops with FBS Decrease

- 10% FBS cells had a higher percentage of S-phase nuclei than 5% and 1% FBS cells
- FBS growth factors may facilitate cells to enter S phase
- S phase entrance indicates propensity to proliferate
- At Day 2, 10% FBS encourages higher levels of proliferation than 5% and 1%

Cell Proliferation Diminishes with FBS Decrease



Exponential Growth Dependency on FBS

- R squared values of 5% and 10% FBS cells indicate exponential growth
- 5% and 10% FBS had cellular doubling rates of 32 hours and 39 hours, respectively
- 1% FBS did not exhibit exponential growth, as denoted by R squared value
- 10% FBS promotes higher cellular doubling rates than 5% and 1% FBS

Cell Numbers Statistically Different Within Days 5 and 7

- One factor ANOVA and Tukey's HSD test revealed statistical relationships between 1%, 5%, and 10% FBS at each time point:

Time point	p<.05 for ANOVA
Day 0	No
Day 2	No
Day 5	Yes
Day 7	Yes

HSD Test Pairings: Day 5	Statistically Different
1% and 5% FBS	Yes
1% and 10% FBS	Yes
5% and 10% FBS	Yes
HSD Test Pairings: Day 7	**Statistically Different**
1% and 5% FBS	Yes
1% and 10% FBS	Yes
5% and 10% FBS	Yes

Cell Numbers Depend on FBS Concentration

- FBS concentration effect on cell number evident after day 2
- Within days 5 and 7: 1%, 5%, and 10% FBS cell numbers were statistically different from one another
- For both days 5 and 7: 10% FBS condition produced highest cell count, followed by 5% and 1% FBS
- For 7 day incubations, 10% FBS promotes cell proliferation more than 5% and 1% FBS

Anti-PCNA Assay Supports Cell Proliferation Results

- For 1%, 5% and 10% FBS--as FBS levels increased:
  - Percentage of S-phase nuclei (Anti-PCNA Assay) increased
  - Cell numbers increased at days 5 and 7
  - Cell doubling times decreased
- Cell numbers and proliferation rates are related to number of dividing cells
- Anti-PCNA confirms that 10% FBS leads to higher cell numbers and proliferation rates by promoting more cells to enter the cell cycle

HDF Cell Constraints: Ethanol Toxicity and FBS Concentrations

- Live/Dead Assay revealed decreased cell viability with increased ethanol levels
- Proliferation Experiments:
  - 10% FBS promotes more cells to enter S-phase than 5% and 1% FBS
  - At days 5 and 7, 10% FBS leads to higher cells numbers than 5% and 1% FBS
  - 10% FBS leads to a smaller cell doubling time than 5% and 1% FBS