Research Topics and Seminar (501)

Presentation and Discussion of the Paper:

Viable offspring derived from fetal and adult mammalian cells

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Outline

- Introduction
- The paper
- Aim
- Methods
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- Conclusion

Introduction

- Dolly:
  - A cloned female sheep.
  - Born in July 5th, 1996, Scotland.

- Why is it special?
  - The first mammal to be cloned from an adult somatic cell.

The Paper

Viable offspring derived from fetal and adult mammalian cells

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Aim

- To investigate whether cellular differentiation to a specific stage involves an irreversible genetic modification.

- To investigate whether normal development is possible when donor cells derived from fetal or adult tissue are induced to exit the growth cycle and enter the G0 of the cell cycle before nuclear transfer.

Methodology

- Cells were obtained from the mammary gland a 6-year-old Finn Dorset female sheep in the last phase of pregnancy.

- Diploid donor cells were induced to become quiescent by reducing concentration of the serum in the medium. Cells were arrested in G0.

- To confirm that the cells entered G0, cells were stained with antiPCNA/cyclin antibody.

Methodology

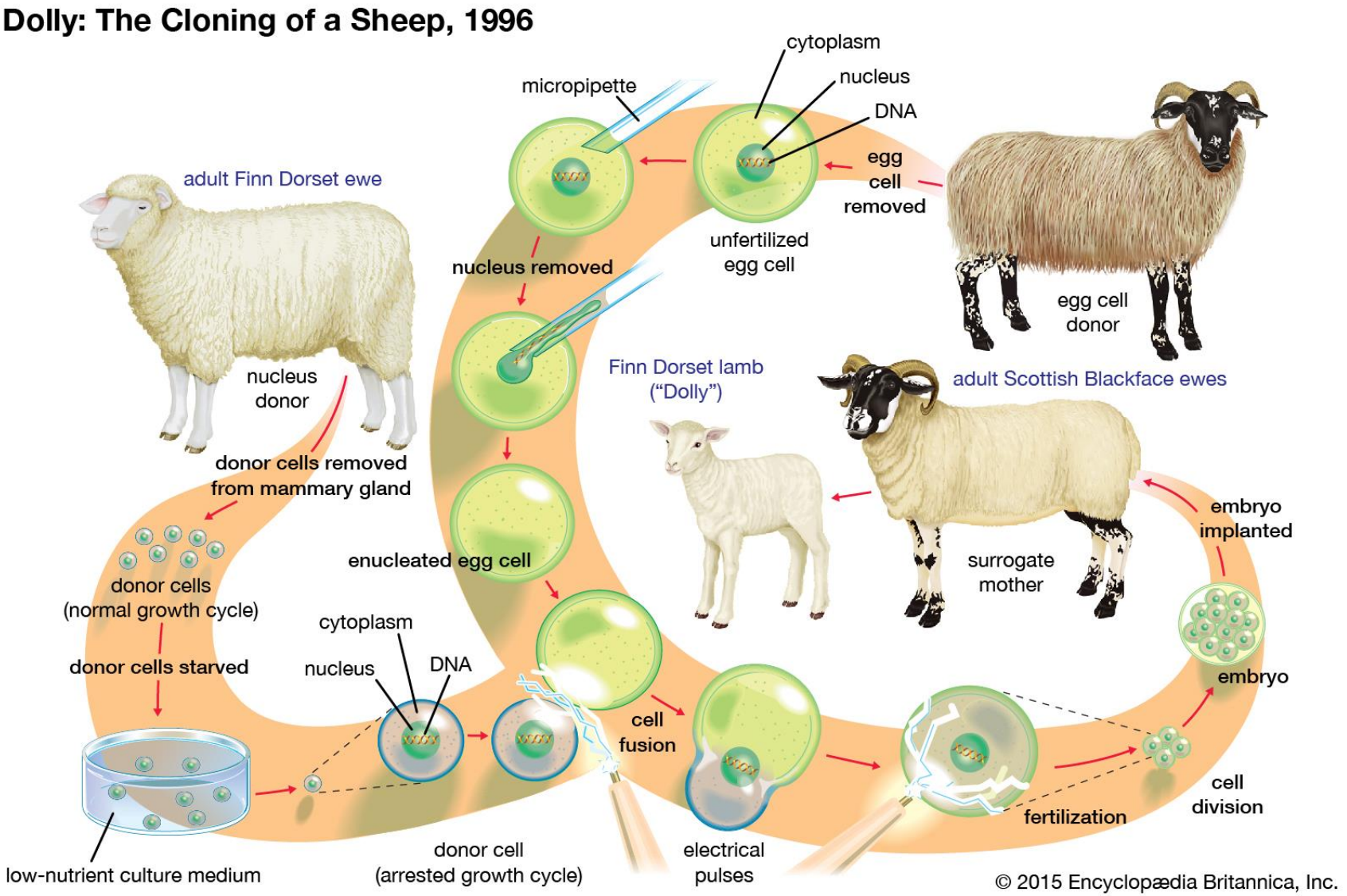
- Oocytes recovered from a female Scottish Blackface after 28 to 33 hours of GnRH injection and were enucleated as soon as possible.
- Fusion of the donor cells to the enucleated oocytes and activation of the oocytes was induced by electroporation.
- Reconstructed embryos were cultured in ligated oviducts of sheep.

Methodology

- Fetal development was monitored in a 2 weeks period using ultrasonic sound.

- After birth, microsatellite analysis was done to compare DNA from lambs and recipient ewes using four polymorphic ovine markers.

Methodology



https://www.britannica.com/science/somatic-cell-nuclear-transfer

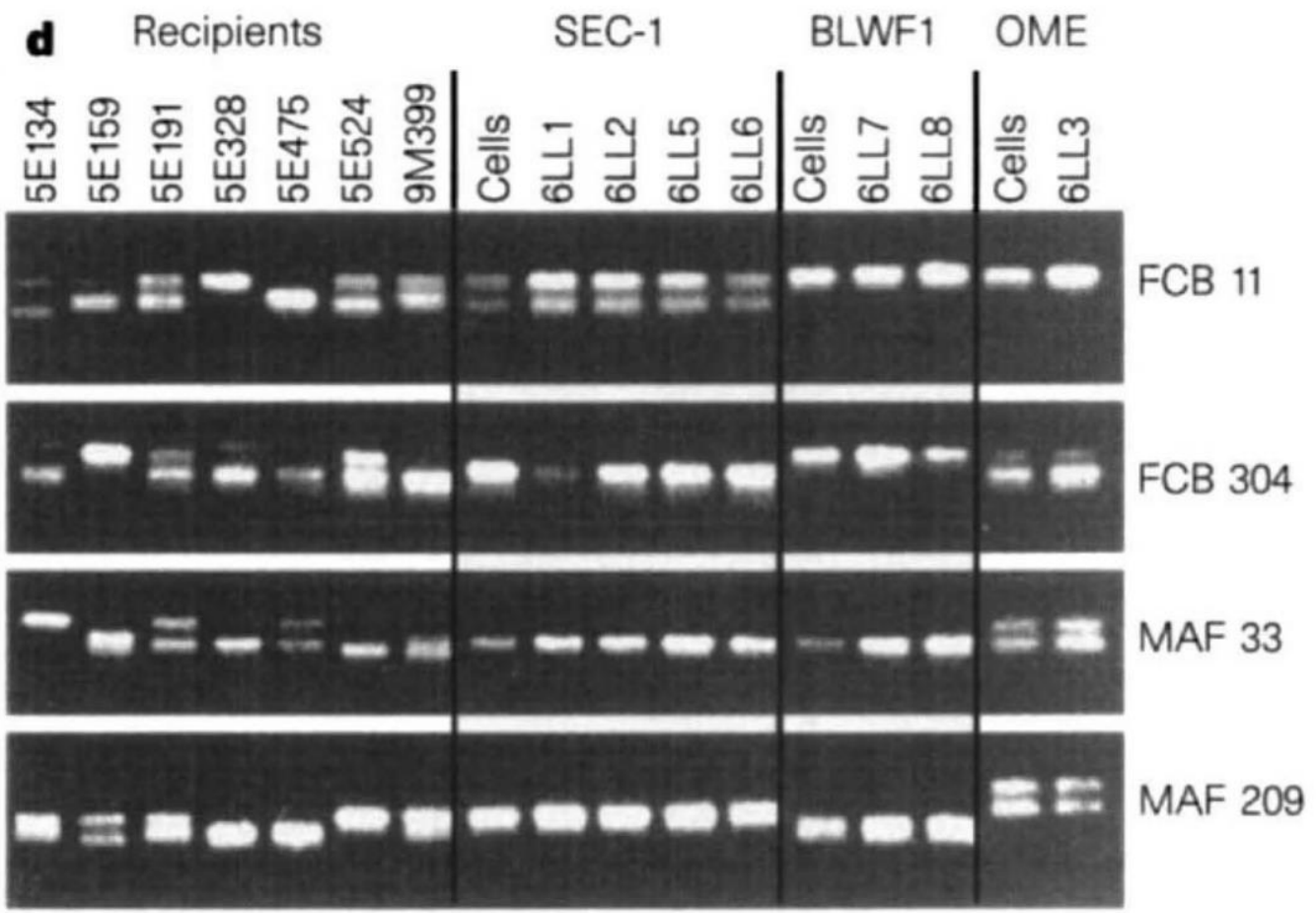
Results

- Ultrasound scanning detected 21 fetuses. Although further scans detected fewer and fewer fetuses. In total, 62% of fetuses were lost.

- 8 ewes gave birth to live lambs. All newborn lambs had morphological characteristics of the donor nucleus and not the donor oocytes.

- DNA microsatellite analysis showed similar results, polymorphic loci showed that lambs were derived from donor cell populations.

Results



(OME). **d**, Microsatellite analysis of recipient ewes, nuclear donor cells and lambs using four polymorphic ovine markers[22]. The ewes are arranged from left to right in the same order as the lambs. Cell populations are embryo-derived (SEC1), fetal-derived (BLW1), and mammary-derived (OME), respectively. Lambs have the same genotype as the donor cells and differ from their recipient mothers.

Results

Table 2 Delivery of lambs developing from embryos derived by nuclear transfer from three different donor cells types, showing gestation length and birth weight

Cell type	Breed of lamb	Lamb identity	Duration of pregnancy (days)*	Birth weight (kg)
Mammary epithelium	Finn Dorset	6LL3	148	6.6
Fetal fibroblast	Black Welsh	6LL7	152	5.6
	Black Welsh	6LL8	149	2.8
	Black Welsh	6LL9†	156	3.1
Embryo-derived	Poll Dorset	6LL1	149	6.5
	Poll Dorset	6LL2‡	152	6.2
	Poll Dorset	6LL5	148	4.2
	Poll Dorset	6LL6‡	152	5.3

* Breed averages are 143, 147 and 145 days, respectively for the three genotypes Finn Dorset, Black Welsh Mountain and Poll Dorset.
† This lamb died within a few minutes of birth.
‡These lambs were delivered by caesarian section. Overall the nature of the assistance provided by the veterinary surgeon was similar to that expected in a commercial flock.

Discussion

- While number of fetuses kept decreasing was most probably due to fetal loss, it may also be due to misdiagnosis.

- Although cells used for nuclear donation that resulted in cloning dolly were from adult cell mammary gland, cell type was unknown.

Conclusion

- It was successfully confirmed that differentiation of cells didn’t involve an irreversible modification of genetic material required for development.

Thank you