Embryo splitting

KSR

Introduction

- It was discovered by HOLSTEIN CALVES in 1982
- Mammalian embryo splitting has successfully been established in farm animals.
- Embryo splitting remains the most effective and rapid method of increasing the number of offspring obtained from an embryo transfer program.
- Artificial twinning is also called embryo cloning or embryo splitting.
- Embryo splitting is safely and efficiently used for assisted reproduction in several livestock species.
- Artificial twinning is really a technological advancement on embryo transfer, which has been used in the cattle industry for many years.
- In this process, a cow with desirable traits is treated with hormones so that she super-ovulates, producing many eggs at one time.

- The eggs are fertilised and then flushed out of the uterus of the biological mother and placed in surrogate mothers
- In the mouse, efficient embryo splitting as well as single blastomere cloning have been developed in this animal system for the first time.
- In non-human primates embryo splitting has resulted in several pregnancies
- Human embryo splitting has been reported recently.

Definition

- **Embryo splitting** or splicing may refer to: when spontaneous, the natural way in which identical twins are formed. when artificially induced, this method is considered as method of cloning
- The term embryo splitting refers to the formation of twins or multiples through artificial microsurgical splitting of an embryo. The cells obtained using this method can each develop into a complete organism in an appropriate environment.

Examples

- In sheep, 36% of embryos split as 2- and 4-cell embryos developed to term following transfer to recipient females
- In cattle, embryos split into blastomeres at the 4-cell stage could further develop to term giving rise to multiple monozygotic healthy calves
- In the mouse it was carried from 2- and 4-cell stage but rarley at 6- and 8-cell embryo stage
- Bisected or biopsied early bovine embryos gave pregnancy rates similar to those obtained from intact control embryos.
- Embryo twinning was therefore proposed for suitable applications under field conditions

Con….

- First and second splitting of embryos has yielded high efficiency rates for blastocysts when compared with the third splitting which did not provide any beneficial advantage and, in fact, resulted in a significantly reduced number of embryos.
- The splitting of rhesus monkey embryos at the 8-cell stage and following their intrauterine transfer resulted in one live-born monkey

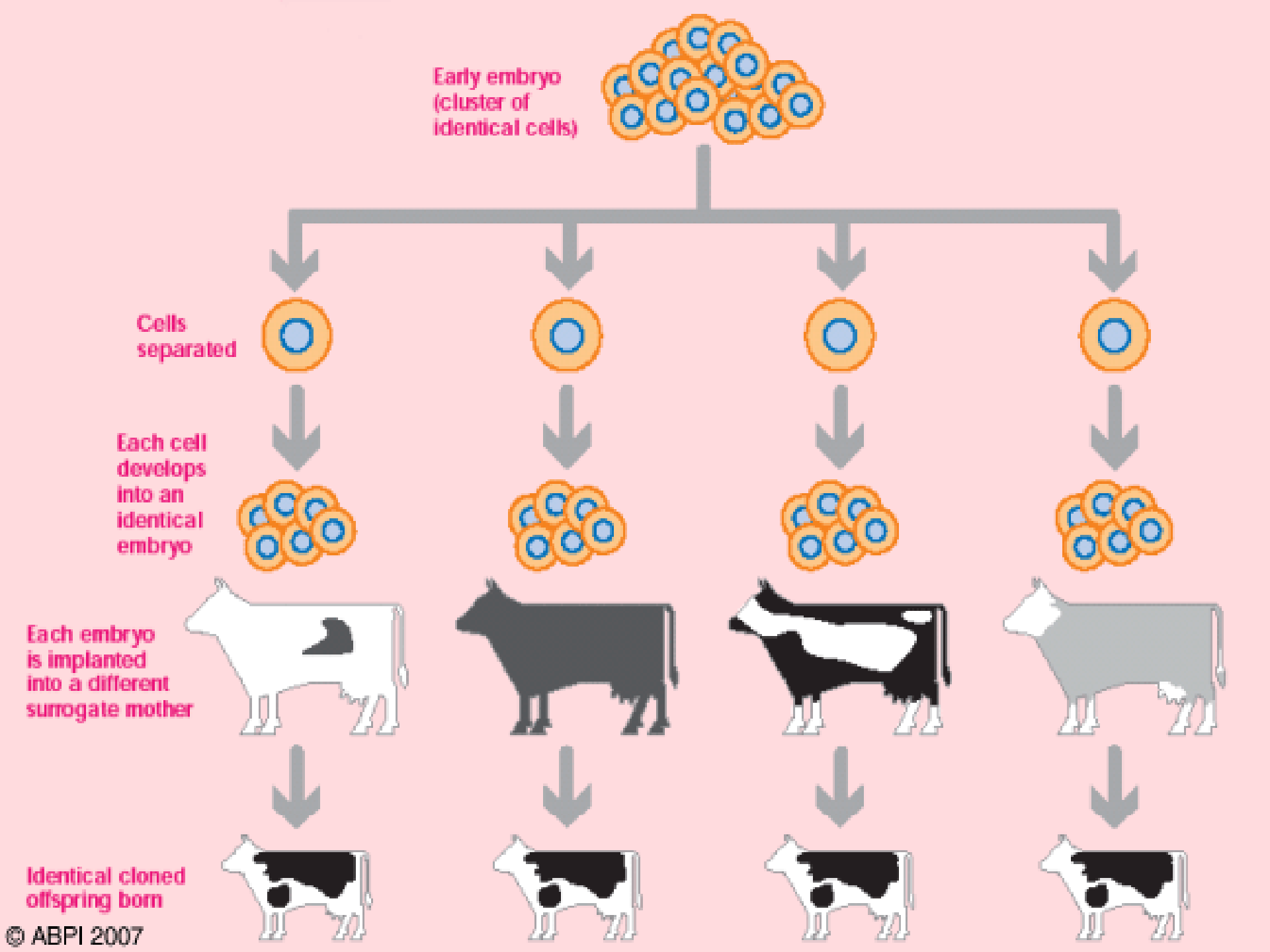
Reason for embryo splitting

- There are two main reasons for splitting the embryos.

1. The first is to *obtain identical twins*, which are very useful for research as well as for certain commercial goals.
2. The second is *to increase productivity*.

Cont….

- Over the past 25 years, mammalian embryo splitting for the creation of genomically identical twins or multiples has advanced to a variety of applications in veterinary and human medicine


Early embryo (cluster of identical cells)
Cells separated
Each cell develops into an identical embryo
Each embryo is implanted into a different surrogate mother
Identical cloned offspring born

Methods

- Dozens or more of procedures for bisection of embryos have been published, from the very complex to the very simple.
- Most of these procedures consist of two stages: immobilizing the embryo and bisecting it.

- The embryo was collected from the female animal or human
- The embryo having cells from 2 to 10 or during blastocyst stage
- It consists of trophoblast and inner cell mass (ICM)

Conti….

- Trophoectoderm is a single layer which lining the inner blastocoel (a fluid filled cavity)
- It is a part of placenta during the latter development
- This mass of cells which latter develops in to a actual embryo
- In embryo splitting this mass in to 2 or more than 2 mass for new embryo formation

- After splitting they were maintained under culture medium for further development and utilized for IVF or normal development
- Spitted cells are immersed in Bovine Serum Albumin (BSA) or Hypertonic sucrose
- High osmotic strength enters into zona pellucida of the embryo. Cells gets negative charge
- Then transfer in to cell culture medium
- Then petridish containing standard cell culture medium

Embryo - positive charge

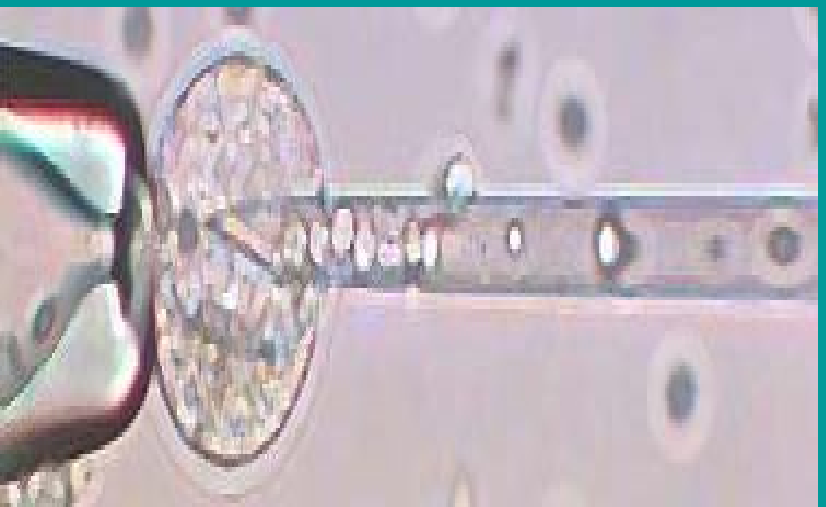
petridish – negative charge

- Due to electrostatic interaction between embryo and petridish
- The embryo stick to the bottom layer of the petridish
- Embryo placed at the stage of the microscope

TWINNER SYSTEM

- TWINNER SYSTEM
- It consist of an microscope and a micromanipulator
- MICROSCOPE Commonly used to work with Samples

e.g inverted microscope , stereoscopic microscope



MICROMANIPULATOR

- combination of tiny glass tools connected to electric motor - driven robot arms linked to a microscope
- Tip of the microtools smaller than head of the sperm

- the embryo cut into 2 halves
- Cells are separated and develop separately
- Embryos are transplanted to surrogate mother or cryopreservation

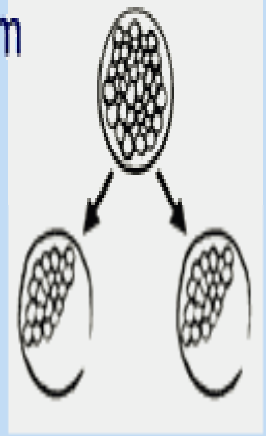
MICROMANIPULATION conti….

- It holds an ultra- sharp splitting blade
- Electric movement is controlled by a variable speed joystick , which permits motion in all 3 axis simultaneously
- Magnification permits accurate morphological assessments of embryo

- Embryo implant and develop with in a month
- Pregnancy monitored by ultrasound
- Embryo develop into offspring

Cloning by Embryo Splitting

Embryo is split to form two half-embryos

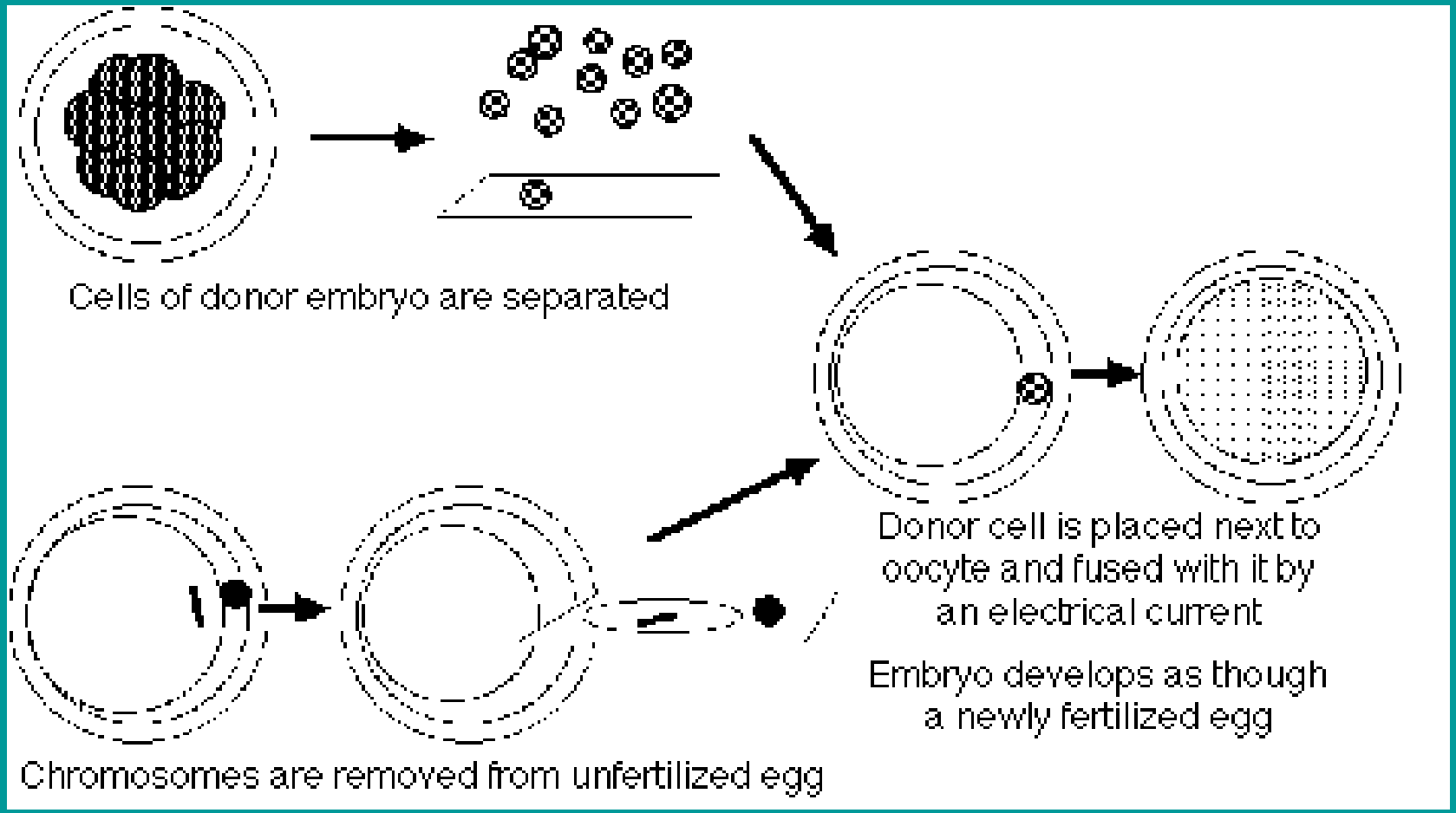


Embryos are transferred to an unrelated surrogate mother

Pregnancy is monitored by ultrasound

Sheep gives birth to identical twins

Simple method



- Pregnancy rates will be high provided that:

(1) the embryo is immobilized without damaging it

(2) the bisection process does not damage too many cells, and

(3) embryos are bisected reasonably symmetrically. Embryos also may be divided into thirds or quarters, but this lowers success rates considerably.


EMBRYO SPLITTING
SIX-CELL EMBRYO
ASSAY
CULTURE IN VITRO
BLASTOCYSTS
CRYO-PRESERVATION
EMBRYO TRANSFER
KARYOTYPE, SEX, PGD, STEM CELLS

Advantages

- All the new ones are genetically identical – they will all have the desired characteristics.
- Organisms that are difficult or slow to breed normally can be reproduced quickly. Some plant varieties do not produce seeds, others have seeds that are dormant for long periods.

- does not kill cell
- no damage to embryo
- fast - can be few hours or less than one hour
- Produce more number of embryos when compared to non- split embryos
- 3-4 cell stage produce high quality embryo
- Increase the rate of progeny

DISADVANTAGES

- need high skilled technician
- 80% accurate
- More than 4 embryos are derived from a single embryo but the quality of the embryo decreased
- Poor quality embryo (too small trophoblast) do not develop

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- If a spitted one is susceptible to disease or changes in environment, then all the clones will be susceptible.
- It will lead to less variation, and less opportunity to create new varieties in the future.

Applications

- Twin formation and delivery can be performed
- Economically viable farm animals can be produced in large number
- Inbreeding can performed very faster if all facilities are viable and ethically acceptable
- Selective breeder race can be maintained
- It has been firmly established in cattle and livestock species
- Multiplication of animals will become an economic factor in animal breeding
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- It should be applicable to human
- To provide child for infertile couples
- Low respond with only a few oocytes produced
- In farm animals, it is well known the embryo split at 2- 6 cell stage produce high quality

CRYOPRESERVATION

- THE preserved embryo can be used
- To produce endangered species
- Organ can be produced when any damage to the developed animal or human