APPLICATION OF REPRODUCTIVE BIOTECHNOLOGIES IN THE NATIONAL GENETIC IMPROVEMENT PROGRAM OF WATER BUFFALOES IN THE PHILIPPINES

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ISO 9001:2000 certified

The Philippine carabao (swamp buffalo)

- An indispensable component of small hold farming in Philippine agriculture
- About 3.3 million carabaos, 99% is raised at backyard farms by smallhold farmers
- Primarily used as draft, and secondary source of meat and milk
- Indigenous buffalo is being transformed as an important source of milk and meat that will benefit millions of smallhold carabao raisers
- PCC is committed to develop the Philippine carabao as a source of milk and meat to improve the general well-being of millions of Filipino farming communities

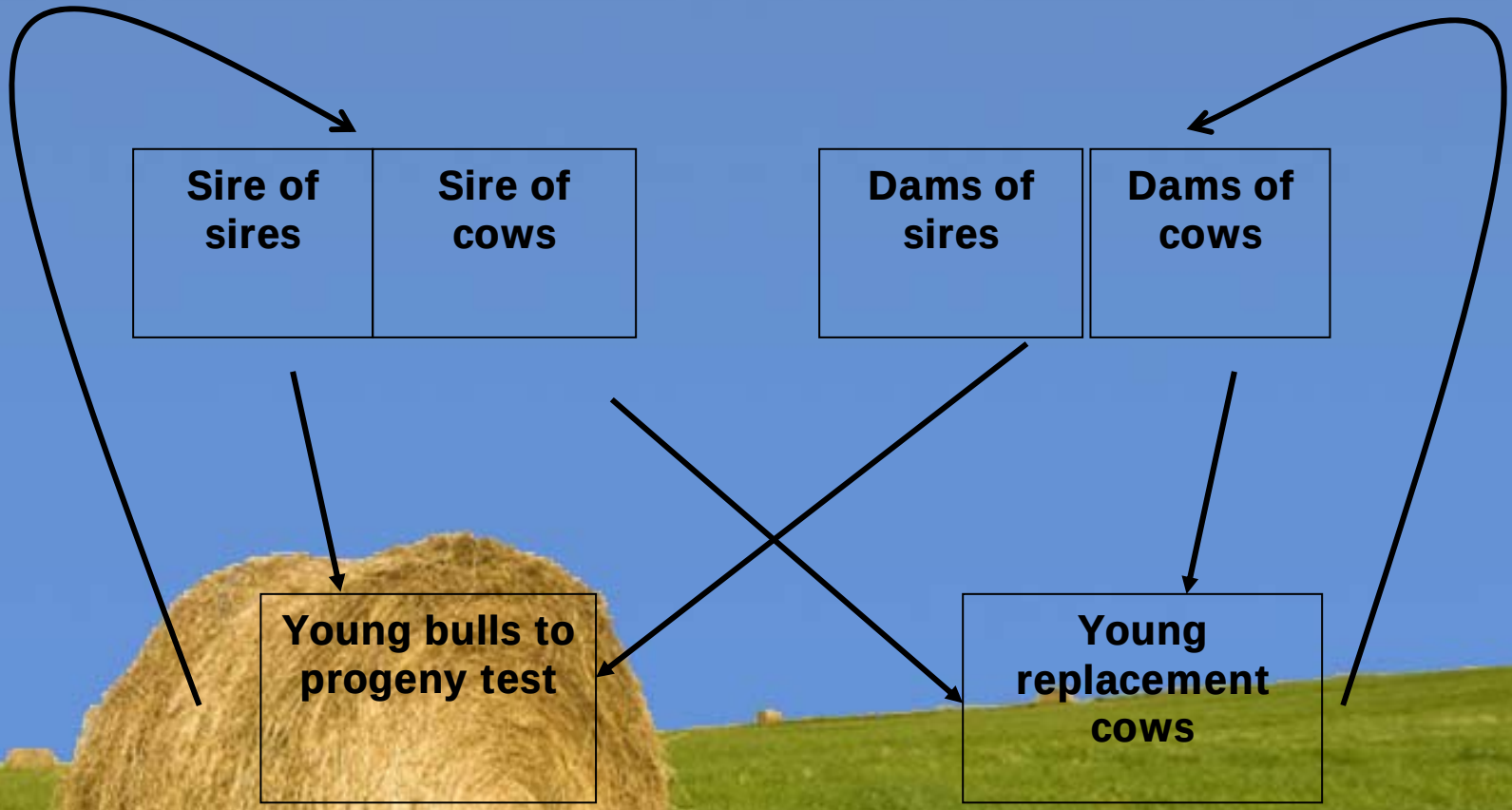
GIP

GENETIC IMPROVEMENT PROGRAM

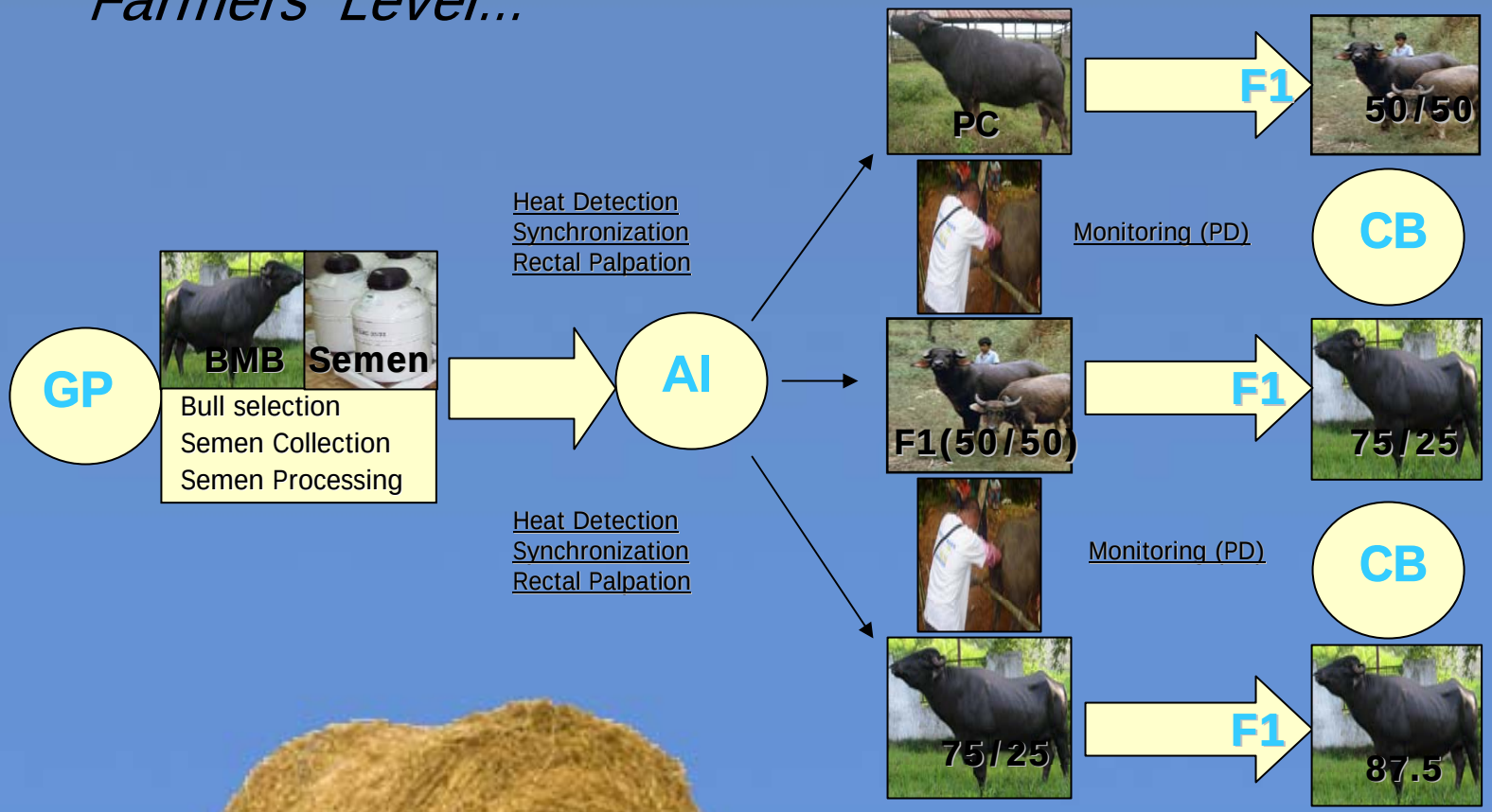
- A science-based approach to address the issues on the need to preserve the existing genetic resource for long-term breeding goals
- The focus is to improve the animal's genetic potential for milk and meat
- The use of reproductive biotechnology to enhance the multiplication of superior germplasm

Gene Pool...

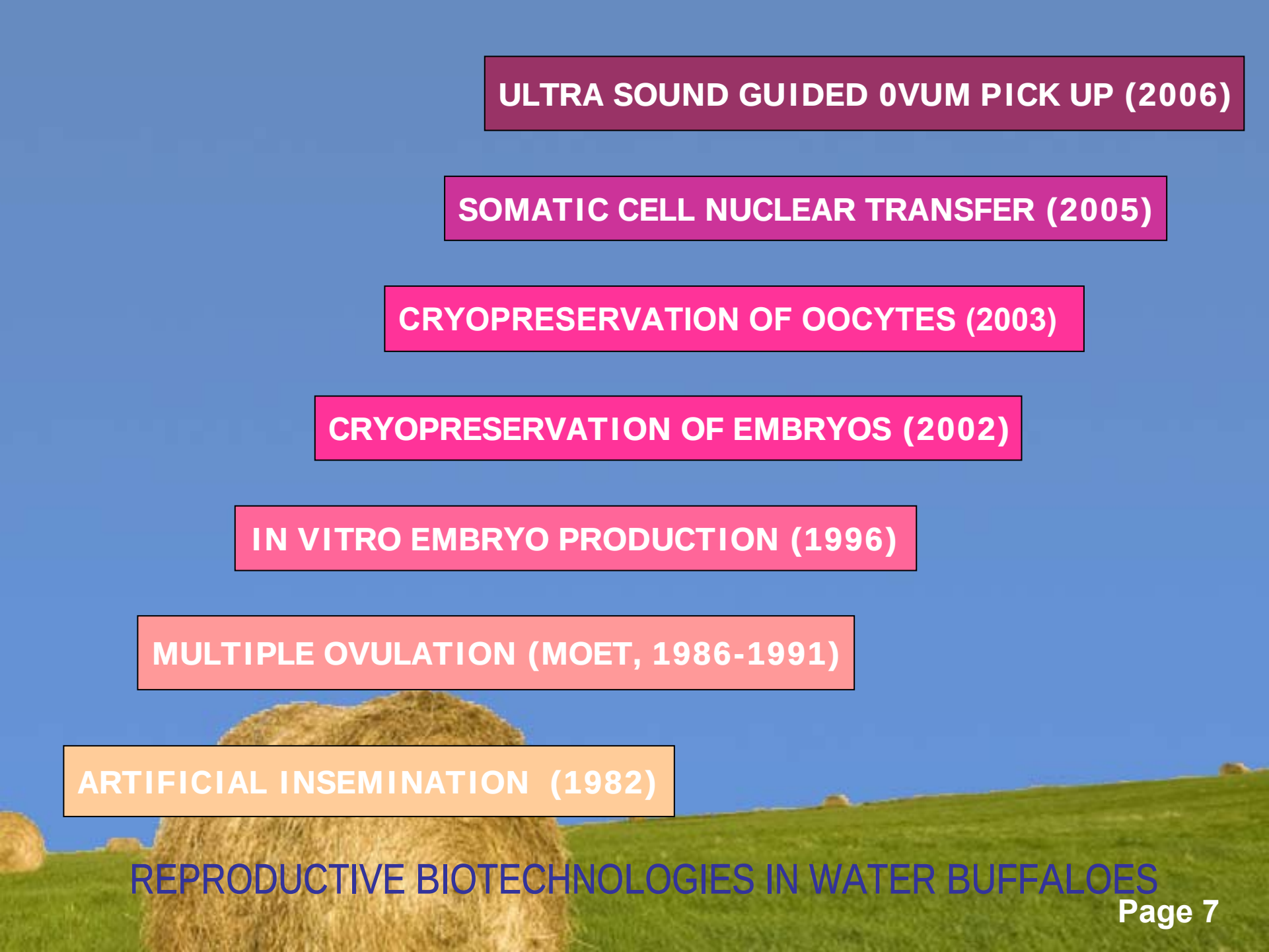
Breeding Scheme for Dairy Buffaloes



Farmers' Level...



With REPRODUCTIVE BIOTECHNOLOGY we can fast track what we envisioned...


ULTRA SOUND GUIDED 0VUM PICK UP (2006)
SOMATIC CELL NUCLEAR TRANSFER (2005)
CRYOPRESERVATION OF OOCYTES (2003)
CRYOPRESERVATION OF EMBRYOS (2002)
IN VITRO EMBRYO PRODUCTION (1996)
MULTIPLE OVULATION (MOET, 1986-1991)
ARTIFICIAL INSEMINATION (1982)

ARTIFICIAL INSEMINATION

- 1982-1992: Strengthening of the PHILIPPINE CARABAO R&D Center was funded by FAO/UNDP & coordinated by PCARRD-DOST
- Strengthening the carabao R&D in terms of manpower and facilities; 6 centers
- Carabao Crossbreeding: Riverine Buffalo x Swamp Buffalo = F1 crossbreds were fertile
- Establishment of Frozen Buffalo Semen Laboratories
- Estrus synchronization technique complements AI
- Demonstration of the viability of crossing the swamp with river buffaloes through economic benefits derived from it

ARTIFICIAL INSEMINATION

- March 27,1992 - PCC was created by RA 7307
- Operational on April 1, 1993 - as attached agency of the Department of Agriculture
- Creation 13 network centers to cover all regions of the country
- Intensified AI program was carried out nationwide
- Present AI scheme: Natural Estrus and Synchronization
- Success rate is 40% in natural estrus, 30% with synchronized estrus
- Localization of AI services with the local government spearheading the program
- Current efforts are directed towards privatizing the AI service by developing the village-based AI technicians

MULTIPLE OVULATION AND EMBRYO TRANSFER (MOET)

- 1986-Research work in MOET was initiated
- Manipulation of ovarian function for *in vivo* production of embryos
- Superovulation of donor, collection of embryos and embryo transfer (ET)
- 1991-first calf was born to Phil carabao surrogate dam by non-surgical transfer
- This pioneering work demonstrated the possibility of using the swamp buffaloes as surrogates for river buffalo embryos

IN VITRO EMBRYO PRODUCTION (IVEP)

- 1996- the first IVM/IVF-derived calves in the country was born
- 2001-Establishment of Satellite Lab in India thru a project funded by PCARRD-DOST
- Development of a simple vitrification technique
- Vitrified embryos were brought into the country for transfer
- April 5, 2002- the first calf from these combined techniques was born

IN VITRO EMBRYO PRODUCTION (IVEP) Present Protocol

IVM

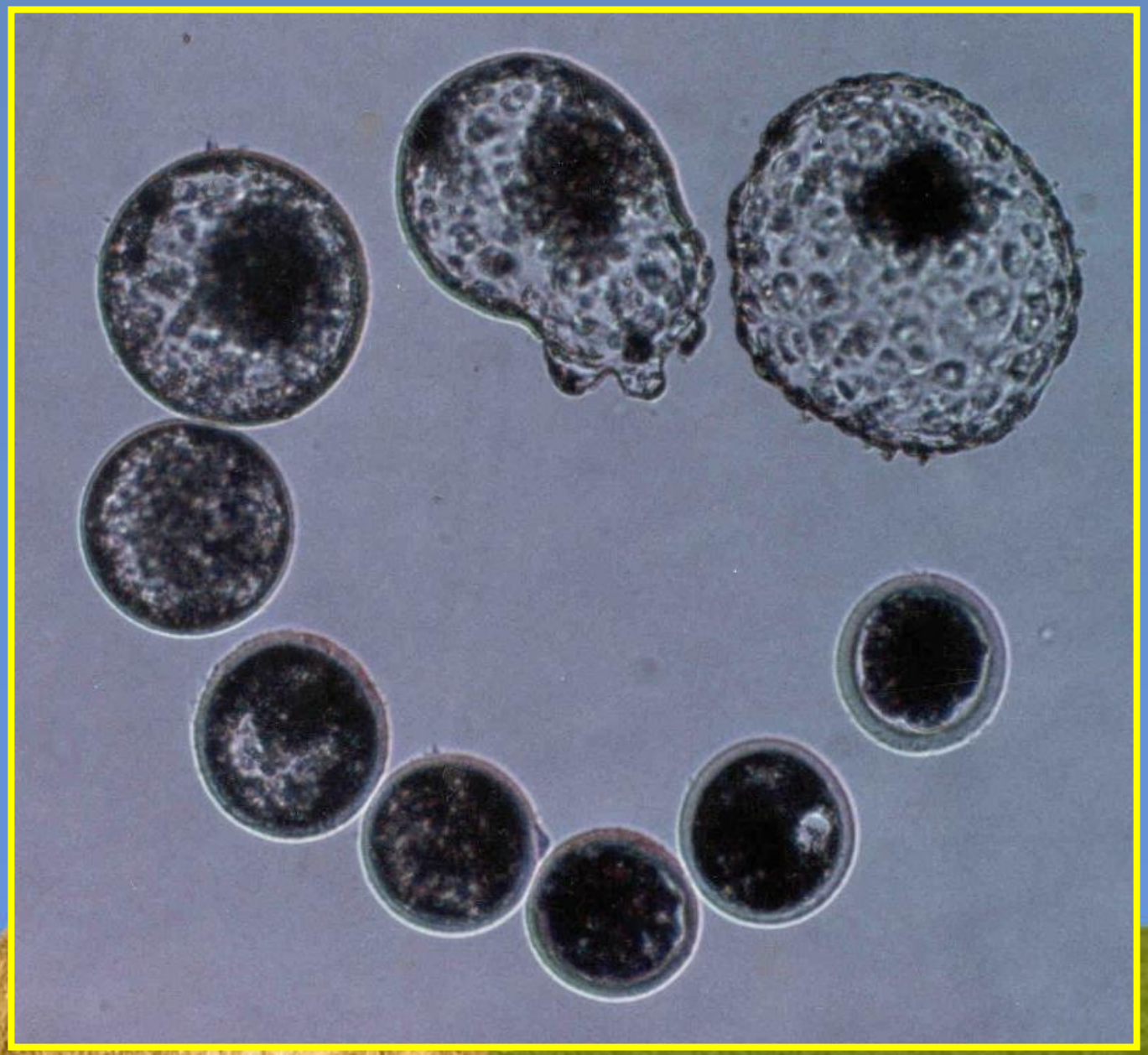
TCM 199 10% FCS
Na Pyruvate 0.2IU FSH
1 µg 17 β estradiol 10 µg EGF
50 µg/ml Gentamicin sulfate
Cultured for 22-24 h at 38.5°C
$C0_2$ 5%, O_2 5%, N_2 90%

IVF

Bracket and Oliphant Medium
Theophylline and Heparin
Percoll Discontinous Gradient
C0-incubation for 12 to 16 h
At the same culture condition

IVC

mSOF + amino acids
for 7-9 days at the same
culture condition



CRYOPRESERVATION OF EMBRYOS

- Vitrification Protocol: Ethylene glycol based VS

Twin calves derived from In Vitro produced/vitrified embryos

5 males: are used for natural mating
5 males: semen donor
2 females: in the Genepool
Others: sold by the farmers

CRYOPRESERVATION OF EMBRYOS

- Success rate: Institutional level=15%

  Field: 5%
- Refinement of the vitrification system that would allow direct transfer of vitrified embryos in the field
- Slow conventional freezing with programmed freezer
- Embryo transfer activities with cryopreserved embryos is being done in fashion similar with AI

CRYOPRESERVATION OF BUFFALO OOCYTES

- Vitrification of buffalo oocytes (immature and matured)
- Ethylene glycol- based VS
- Post thawing survival
- Maturation rate
- Fertilization rate
- Development to blastocyst had been achieved

Cloning

What is Cloning?

- A process of producing identical copies of an individual
- Multiplication of animals by asexual reproduction

Cloning by Nuclear transfer

- Transfer of nucleus from one cell into another hence "Nuclear Transfer"

Embryonic Blastomere Nuclear Transfer (EBNT)

Somatic Cell Nuclear Transfer (SCNT)

Application/Merits of Nuclear Transfer

- Multiplication of animals with inherent genetic/commercial value
- Rescue and propagation of rare and endangered animal species
- Restoration of extinct animal species
- Production of transgenic animals for clinical application in biomedicine

Nuclear Transfer (Embryonic Blastomere)

Oocyte enucleation

Blastomere isolation

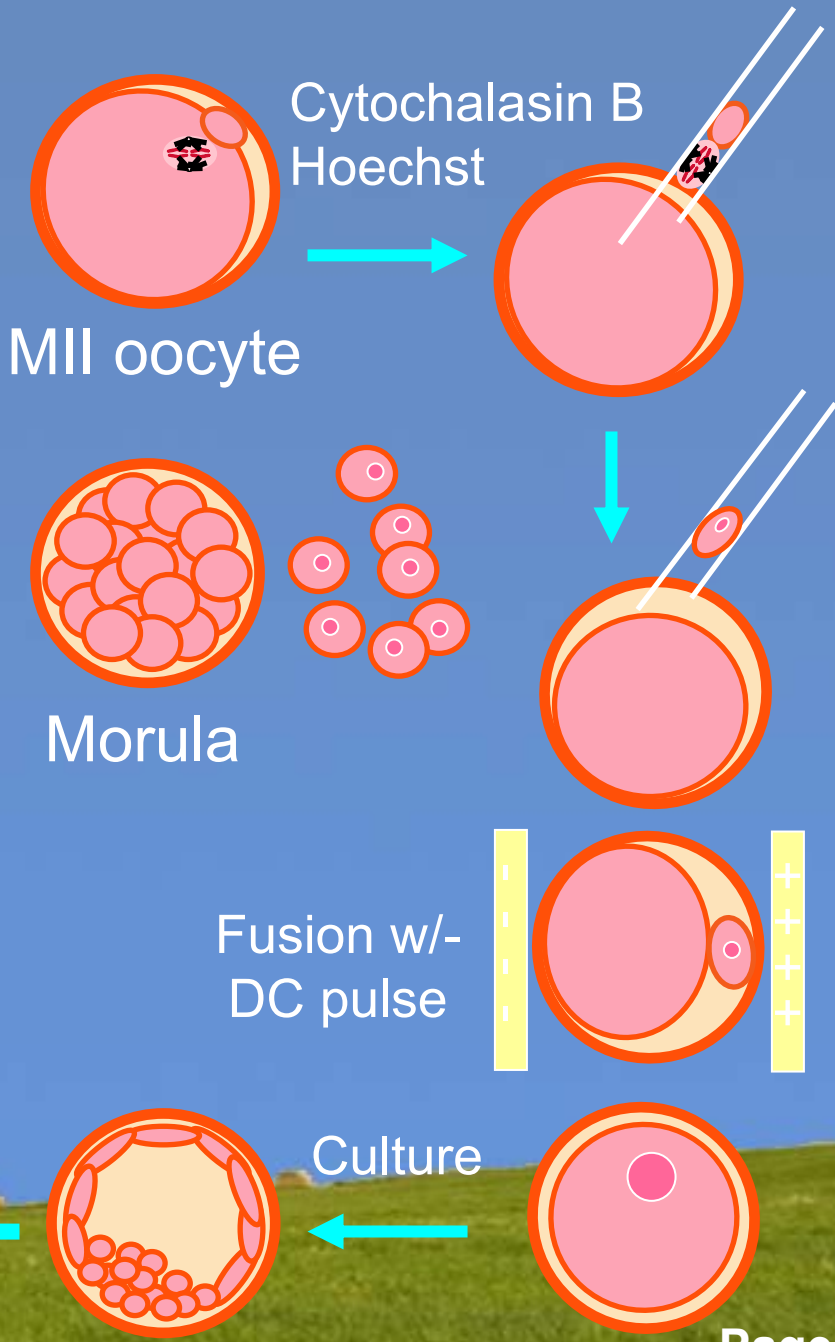
Nuclear transfer

Electrofusion/Activation

A23187 or ethanol + Cycloheximide

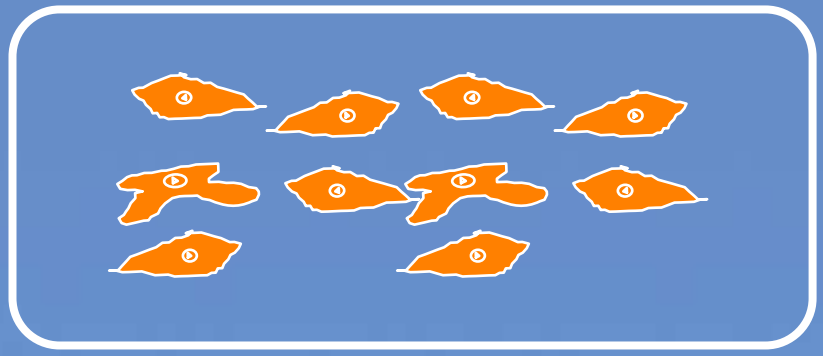
Embryo culture

Embryo transfer



Somatic Cell Nuclear Transfer

What are somatic cells?



Two main types of cells:

1. Germ Cells
   - ovum and sperm cells
   - through sexual fusion will produce a progeny

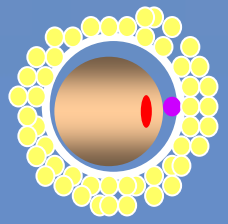
2. Somatic cells- forms the rest of the body
   - help the germ cells to survive and propagate
   - leaves no progeny

♥ Offspring can now be produced from somatic cells

Cloned Animals Produced by Somatic Cell NT

Species	Donor cell	Authors/Researchers
Sheep	mammary gland cell	Wilmut et al.,1997
Cattle	fetal fibroblast	Cibelli et al.,1998
Mice	cumulus cell	Wakayama et al.,1998
Goats	fetal fibroblast	Baguisi et al.,1999
Pigs	granulosa cell	Polejaeva et.al.,2000
Cats	cumulus cell	Shin et al.,2002
Rabbit	cumulus cell	Chesne et al.,2002
Mule	fetal fibroblast	Woods et al.,2003
Horse	adult fibroblast	Galli et al.,2003

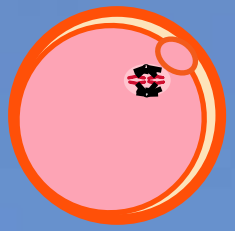
Somatic Cell Nuclear Transfer In Buffaloes



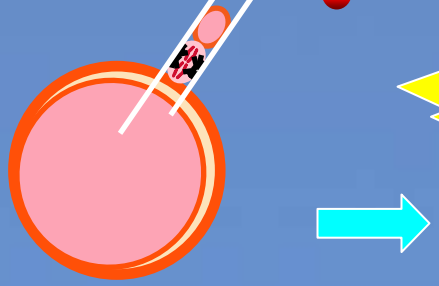
Recipient

Donor (Super Buffalo)

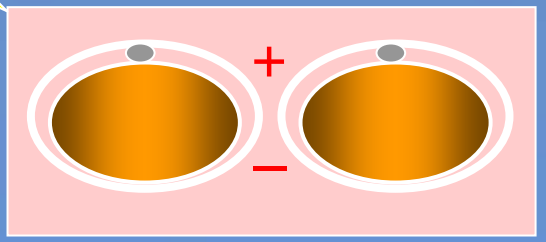
Collection of ear skin sample



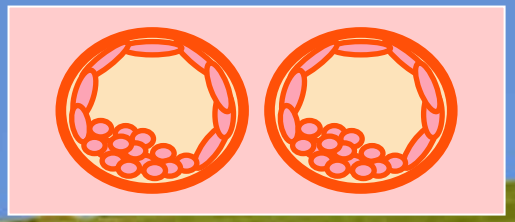
Enucleation of oocytes



Transfer of somatic cells to enucleated oocytes



Electrofusion and culture of reconstructed embryos

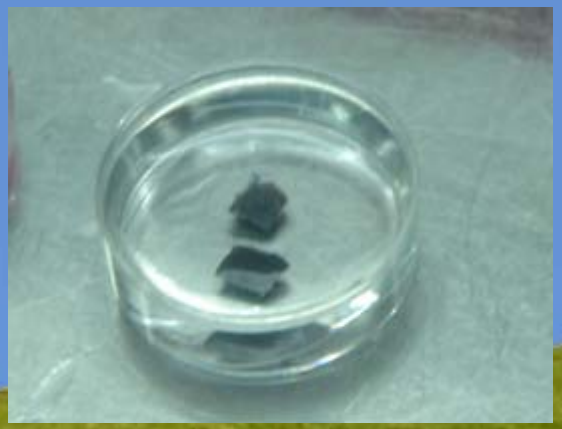


In Vitro Culture of Clone embryos

Transfer of NT embryos to surrogate dams

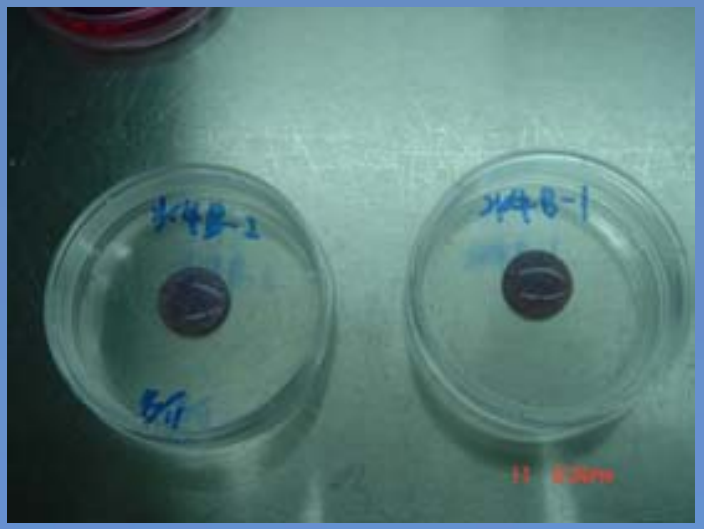
Clones of Superior Buffalo

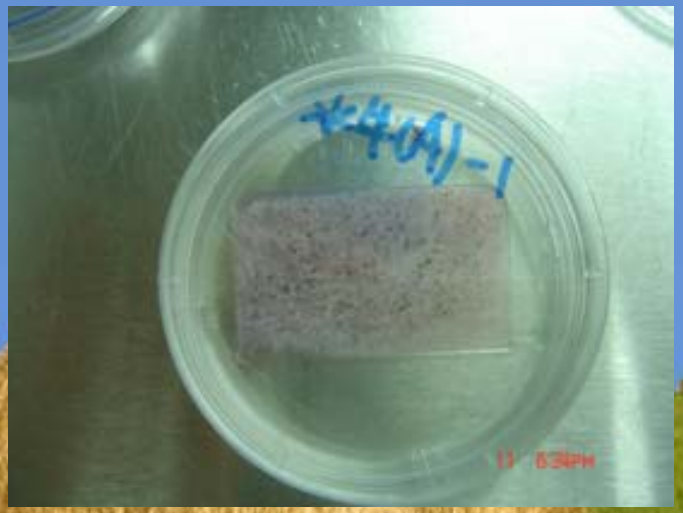
Collection of buffalo ear tissue



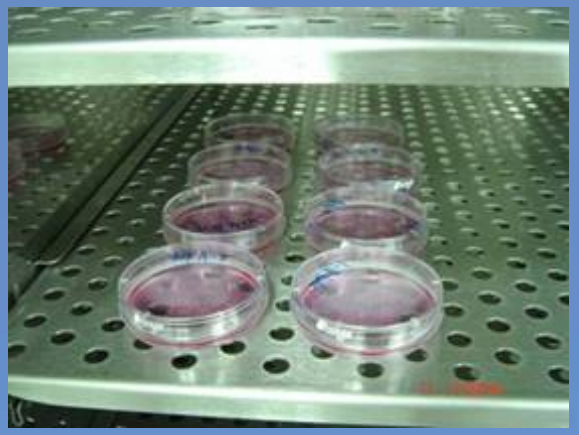
3 mm^3 ear tissue

Cut into small pieces

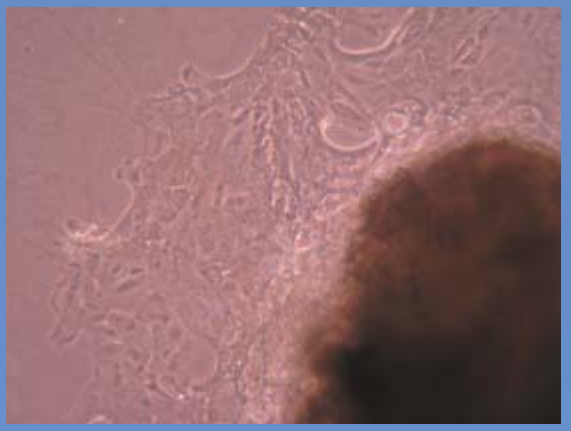




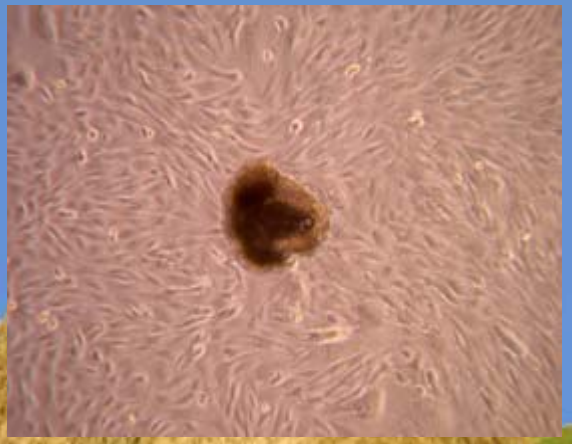
Primary culture of ear explants



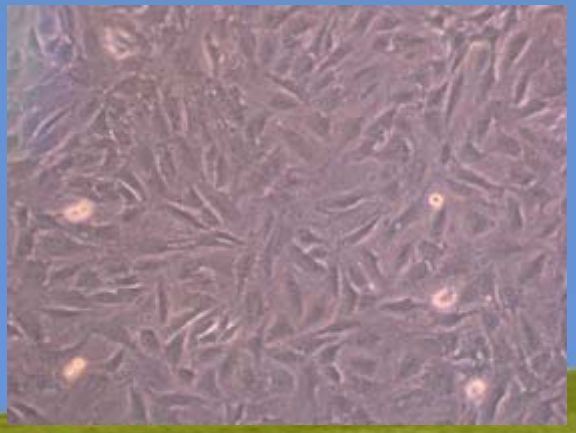
Culture in CO_2 incubator



Cells outgrowth from ear skin explants on day 7



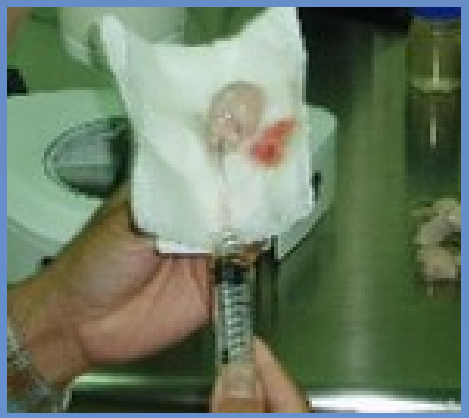
Cells outgrowth from ear skin explants on day 11



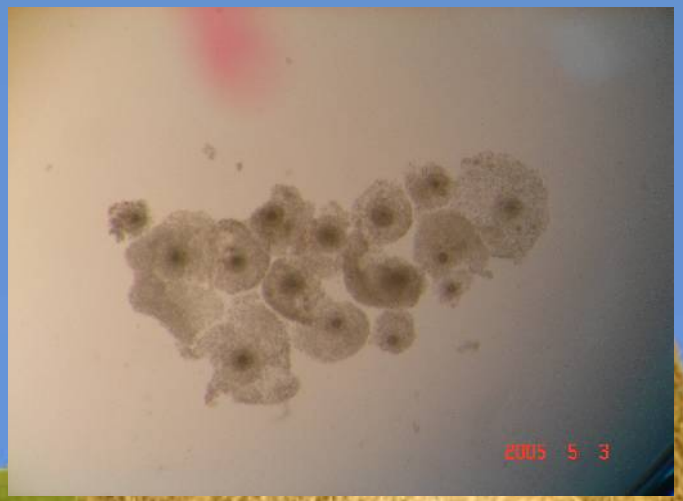
A male buffalo ear fibroblast (passage = 10)

In vitro maturation of buffalo oocytes

Bovine Ovary



Cumulus -OCs recovered by syringe aspiration

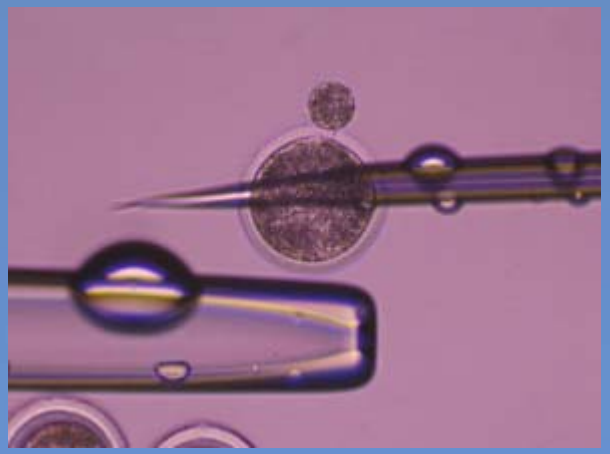


In Vitro Matured Bovine Cumulus –Oocyte complexes

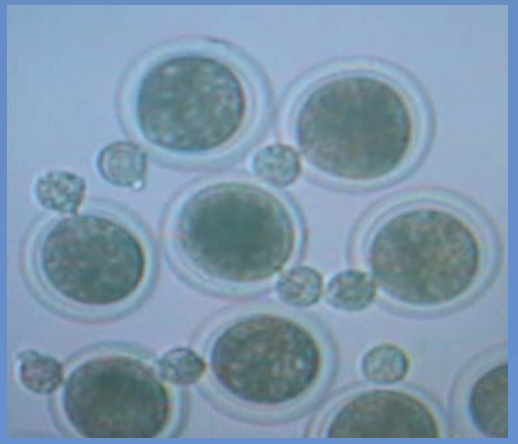
In Vitro Matured Oocyte and removed of cumulus cell

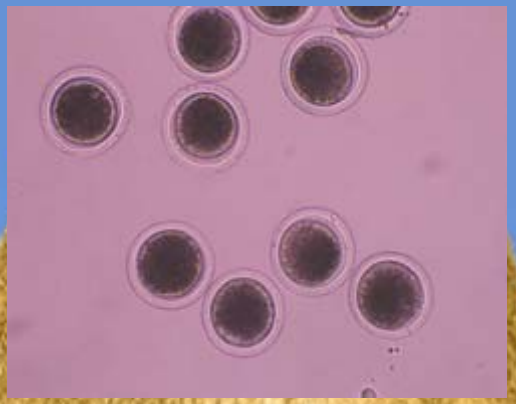
Enucleation and confirmation

Cut the Zona Pellucida



Squeeze to push out the PB and some of ooplasm

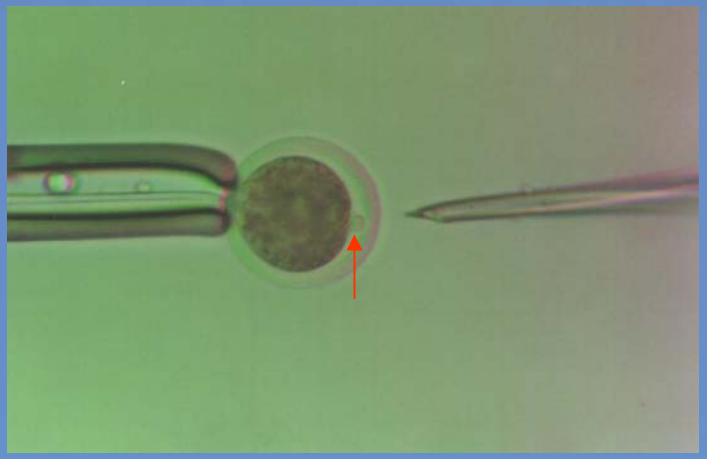


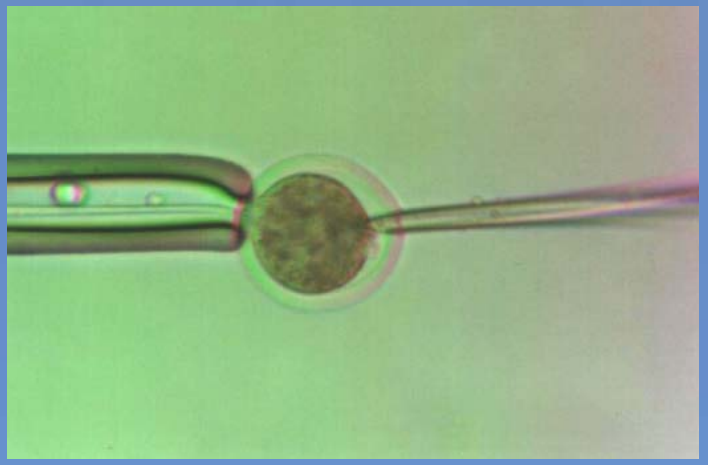


Hoechst 33342

Successful enucleation was confirmed by Hoechst 33342 staining

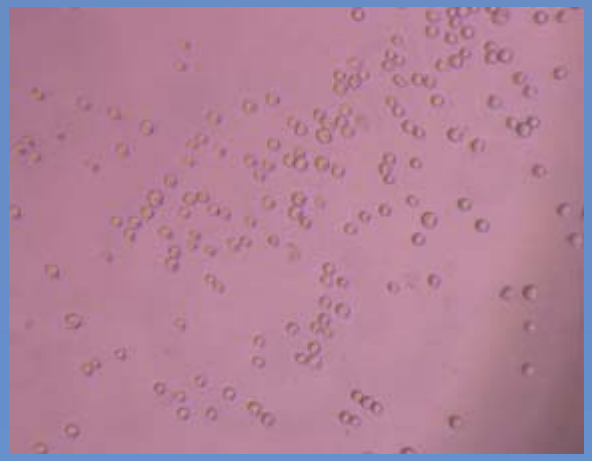
Enucleation





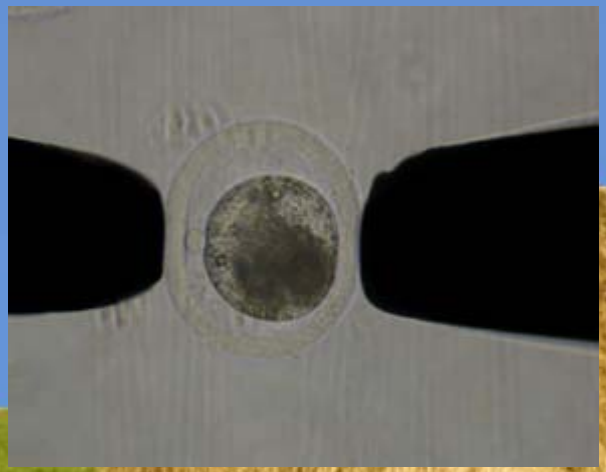
Preparation of enucleated oocyte. The first polar body (arrow) and MII plate with small volume of surrounding cytoplasm were aspirated using a beveled 25-μm outside-diameter glass pipette.

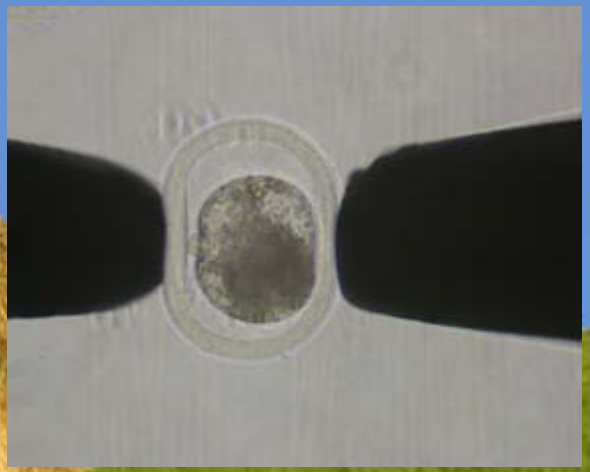
Nuclear transfer and electrofusion



Trypsinization of cells after serum starvation

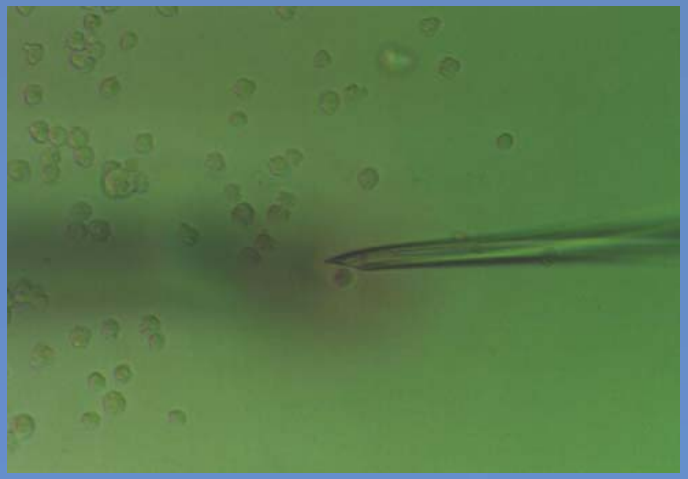
(0.5% FBS in DMEM)

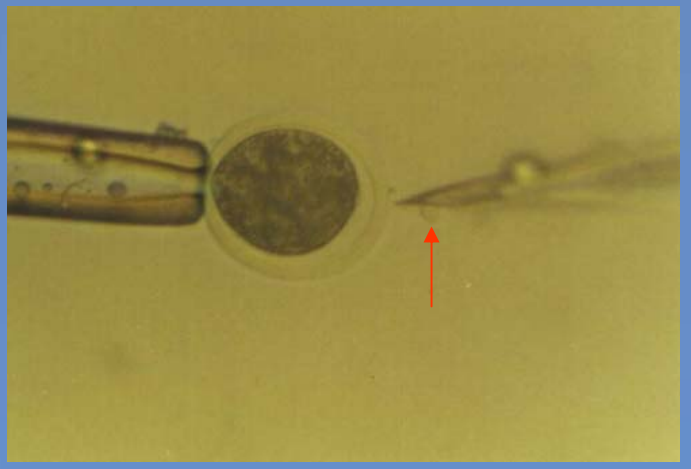


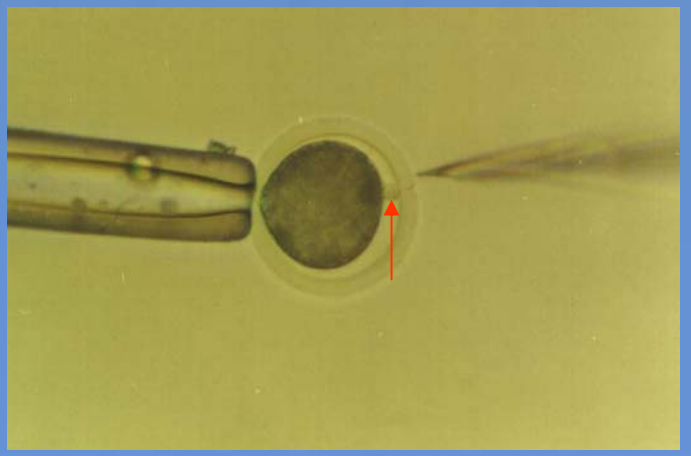


BTX Electro Cell Manipulator 2001

Transfer of donor cell into enucleated oocyte







A donor cell (arrows) was transferred into perivitelline space of enucleated oocyte using a beveled 25-μm outside-diameter glass pipette.

EFFICIENCY of IVEP

	SCNT	IVF
In Vitro Maturation	85	85
Enucleation	75	
Fusion	70	
Cleavage	87	65
Blastocyst Development	20	25-30
No. of transfers made	18	
Pregnancy	-	

Factors affecting efficiency:

- Incomplete reprogramming
- In Vitro Culture system
- Synchronization with recipients

OVUM PICK UP (OPU)

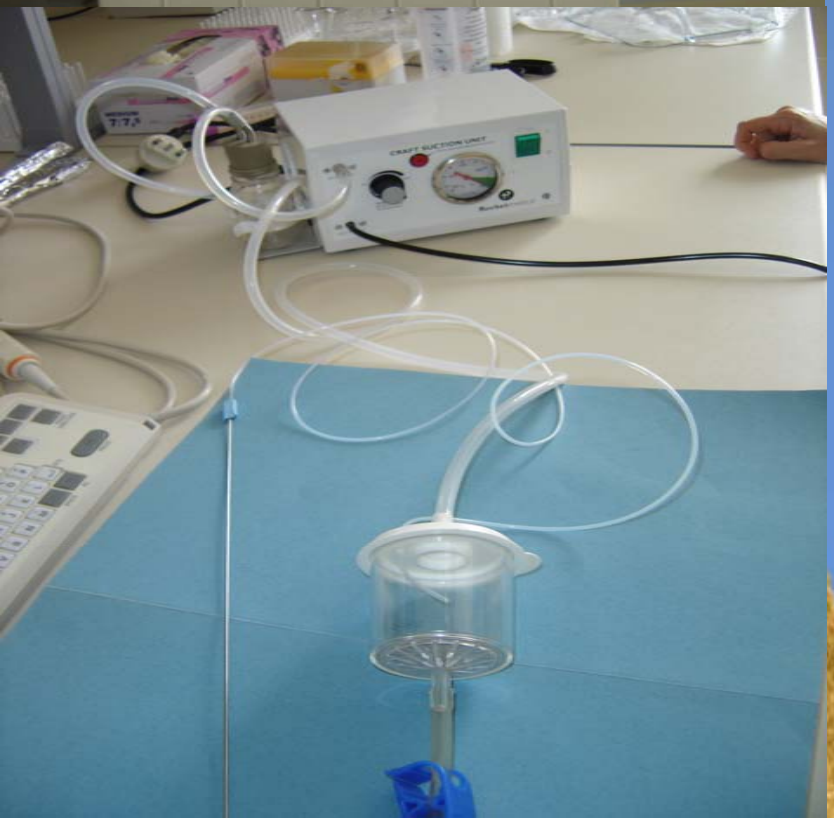
- The most recent technique adopted to propagate superior female germplasm
- Training on OPU in water Buffaloes was conducted under an Italian Expert

PURPOSE OF OPU

- To retrieve repeatedly cumulus-oocytes complexes from animals of high genetic merit
- To generate large number of calves with known production traits
- To shorten the generation intervals in breeding programs

WHY OPU?

- Alternative and competitive to MOET programs
- Feasible to any kind of physiological status
- For unlimited amount of time
- Can be used in prepuberal animals



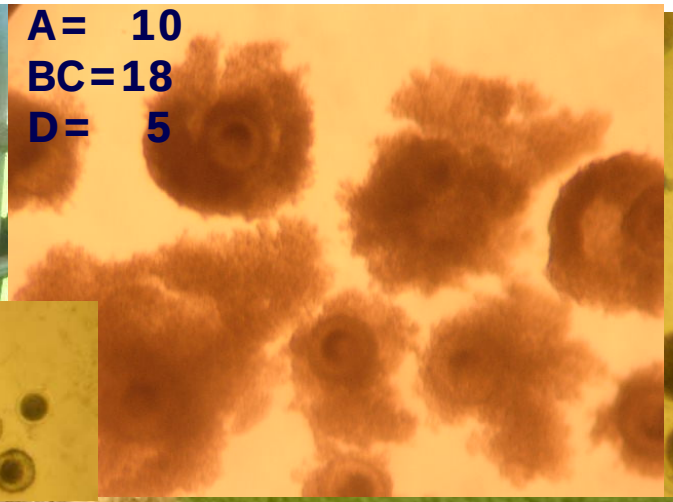
Equipment and supplies needed in the Conduct of OPU

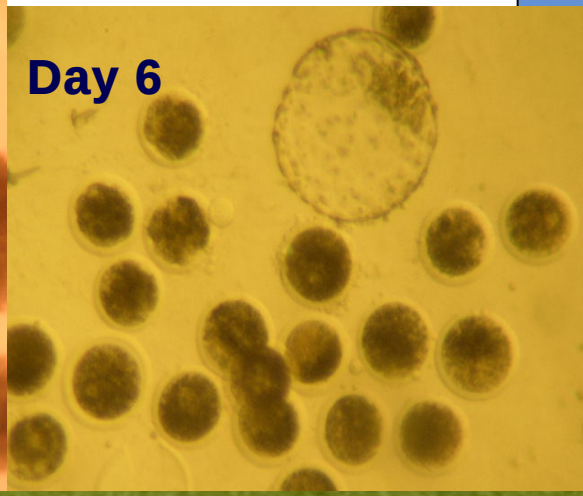
- Ultrasound unit
- 5 MHz sector scanner transducer
- Vacuum pump
- 15 and 50 mL tubes
- EmCon filters
- 17g x 55 cm aspiration needles (adult animals)
- 17g x 32 cm aspiration needles (prepuberal animals)
- Styrofoam containers
- 12 mL syringes
- 18 gauge syringe needles
- Lubricant gel
- Recording sheets
- Plastic sleeves
- Condoms

OPU SCHEME

- **Prepuberal calves (9 mos) – gonadotropin stimulated and unstimulated**

250 IU Serotropin	250 IU Serotropin CIDR	FSH 5+5 mg	FSH 4+4	FSH 3+3=24mg	OPU
Day 0	7	14 Antrin	15	16	17
			12-14 h after last FSH injection Remove CIDR		





Results of OPU investigation

- It is possible to obtain large number of oocytes with good developmental potential from gonadotropin stimulated prepuberal calves
- Without gonadotropin stimulation, only few oocytes were recovered with poor quality and developmental ability from prepuberal calves
- It was demonstrated that pregnant animals (3 months) can be used as donor animals for OPU
- Clearly demonstrated the potential of OPU in enhancing reproductive efficiency of females, reducing genetic interval and accelerating genetic gains in water buffalo

Technical components

1. Retrieval procedure
2. Needle diameter
3. Aspiration vacuum
4. Operator

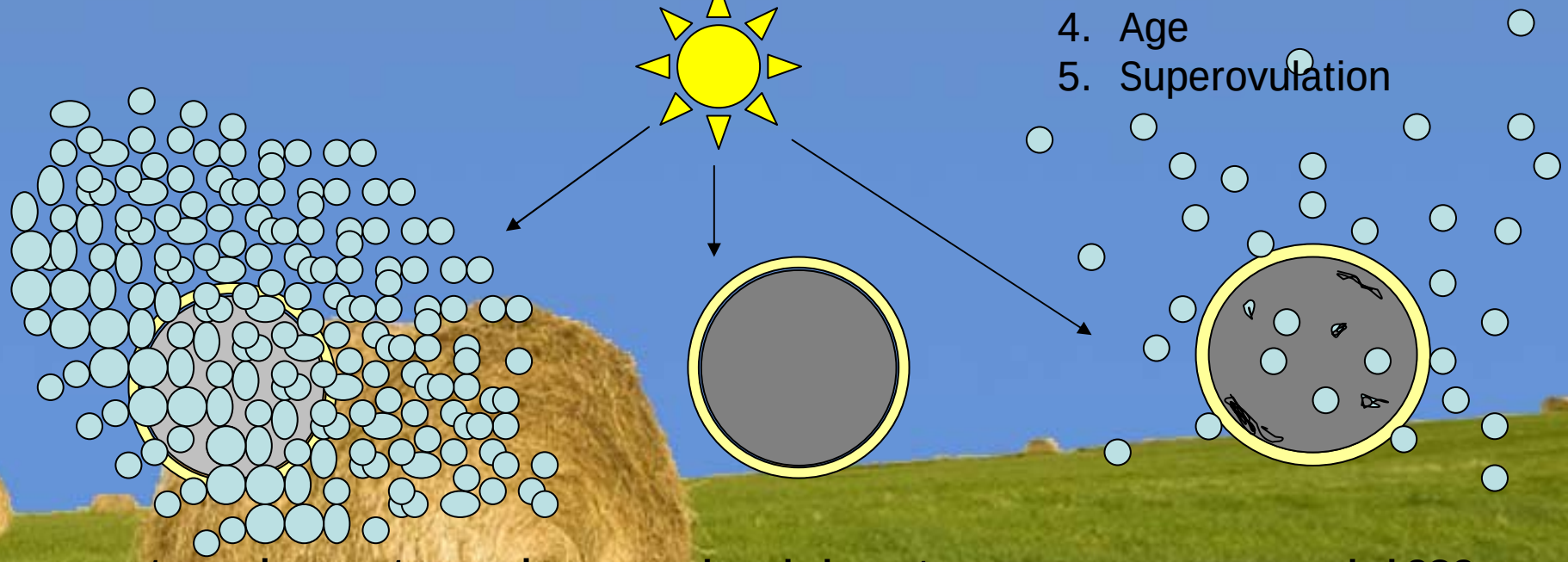
Buffalo

OOCYTE

RETRIEVAL

Biological components

1. Stage of estrous cycle
2. BCS
3. Breed
4. Age
5. Superovulation



compact cumulus oocyte complex **denuded oocyte** **expanded COC**

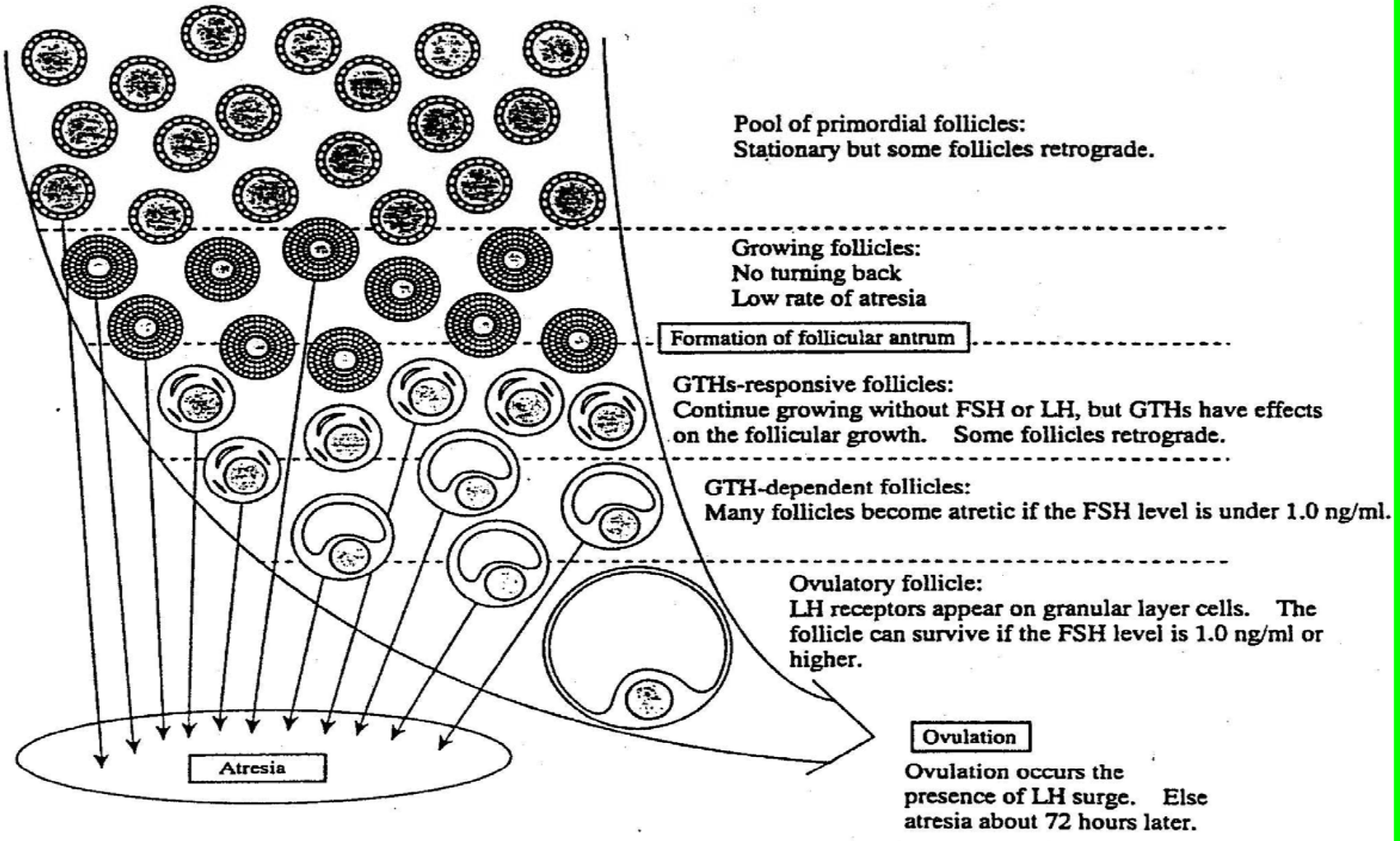
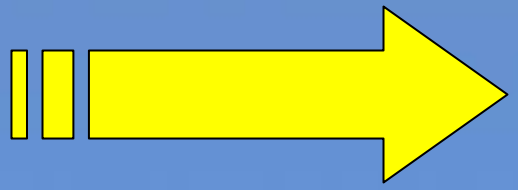


Fig. 24 Model of Follicular Growth in Sheep

Sensitivity of and dependency on gonadotropic hormones vary with the growth of ovarian follicles.

(Scaramuzzi *et al.*, 1993; after slight modification)

Direction of Buffalo R&D




a unique world of
endless
possibilities

MULTI COMMODITY AGENCY

REPRODUCTIVE BIOTECHNOLOGY

ISO 9001:2000
certified

Thank you.....