수정란이식 기술의 미래

임 정 묵
서울대학교 농업생명과학대학

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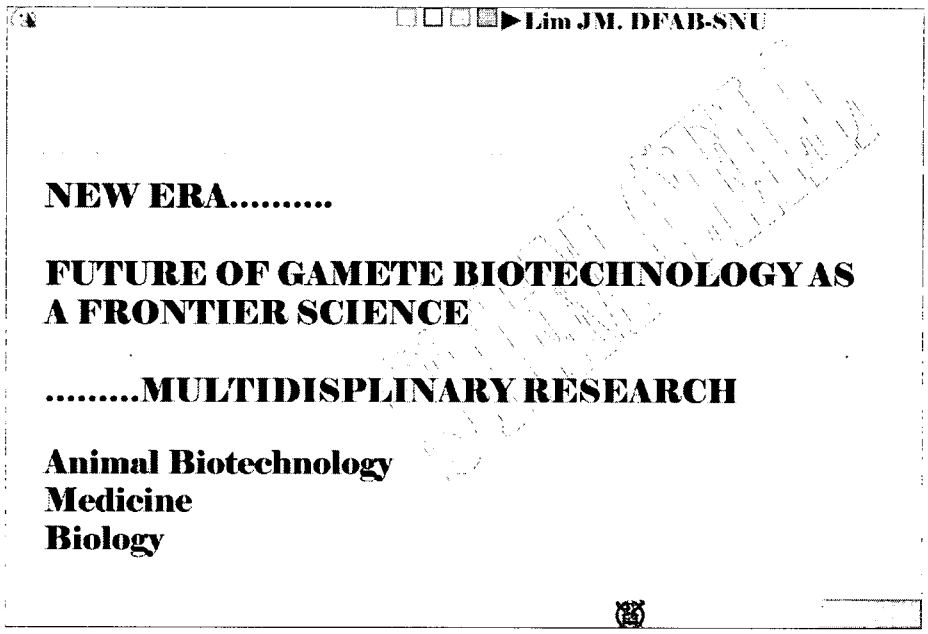
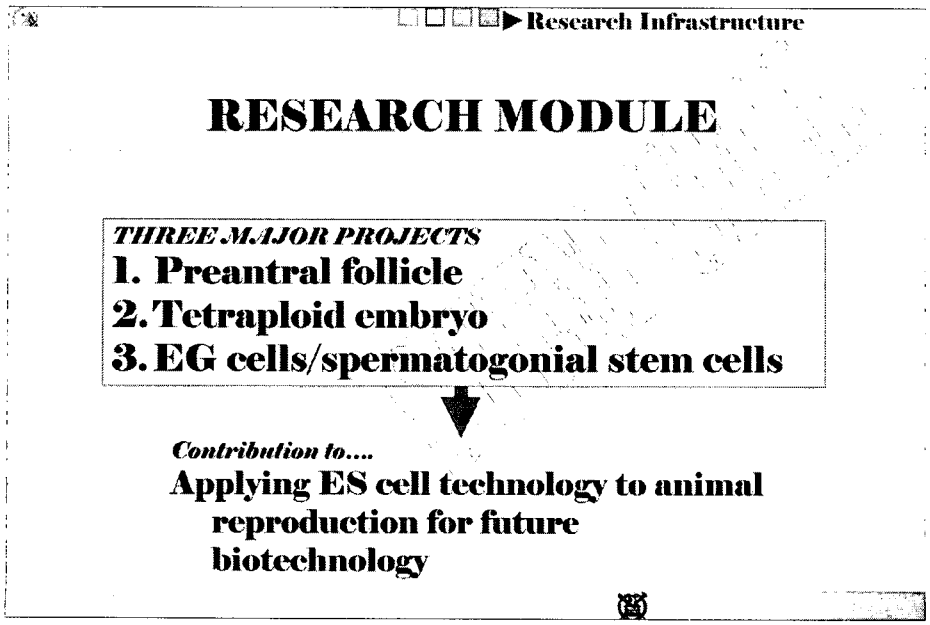




PREANTRAL FOLLICLE CULTURE

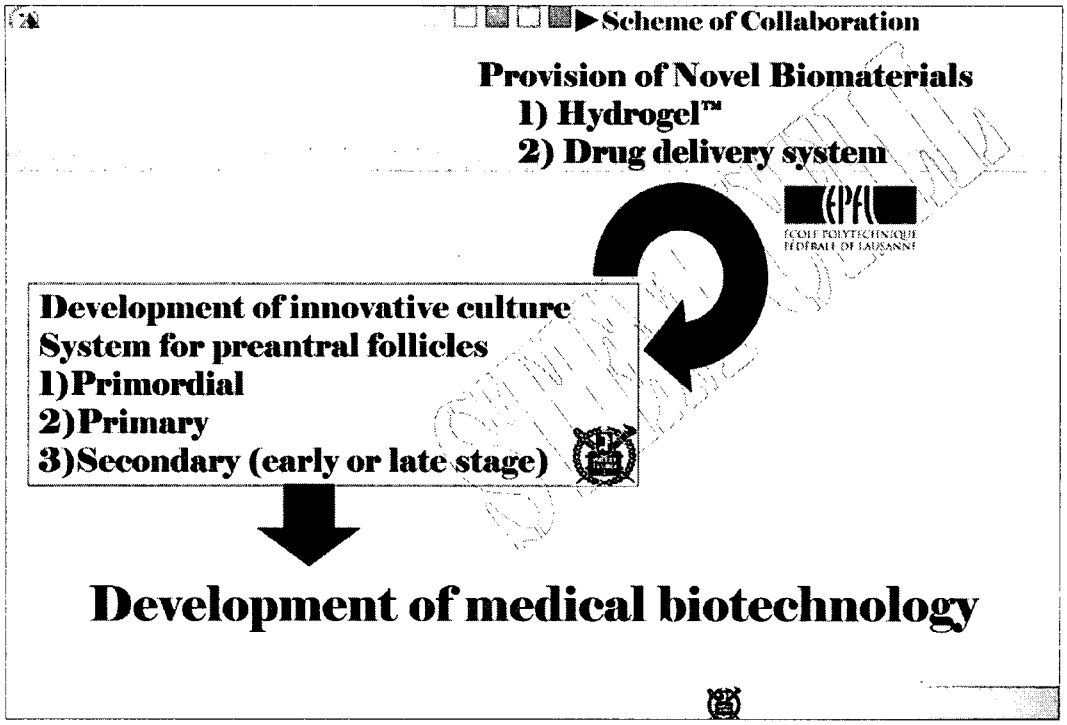
▶ Collaboration of SNU with EPFL

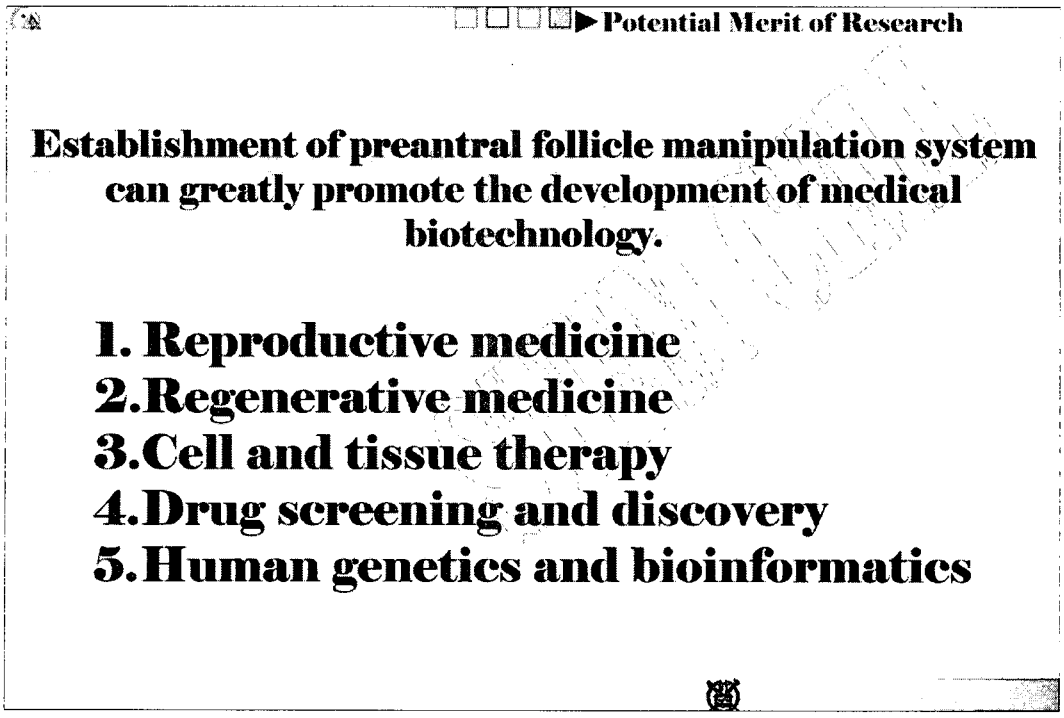
Laboratory of Embryology and Gamete Biotechnology
(Supervised by Dr. Jeong Mook Lim)
Seung Tae Lee, Moon Hwan Choi
Seung Pyo Gong, Gil A Kim
Other graduate students

Launched from November 2004

Laboratory of Regenerative Medicine and Pharmacobiology
(Supervised by Dr. Jeffrey Hubbell)
Jong Eun Ihm
Other research members

ECOLE POLYTECHNIQUE
FÉDÉRALE DE LAUSANNE


Scheme of Collaboration
Provision of Novel Biomaterials
1) Hydrogel™
2) Drug delivery system
EPFL
ECOLE POLYTECHNIQUE FEDERALE DE LAUSANNE
Development of innovative culture System for preantral follicles
1)Primordial
2)Primary
3)Secondary (early or late stage)
Development of medical biotechnology


Potential Merit of Research
Establishment of preantral follicle manipulation system can greatly promote the development of medical biotechnology.
1. Reproductive medicine
2.Regenerative medicine
3.Cell and tissue therapy
4.Drug screening and discovery
5.Human genetics and bioinformatics

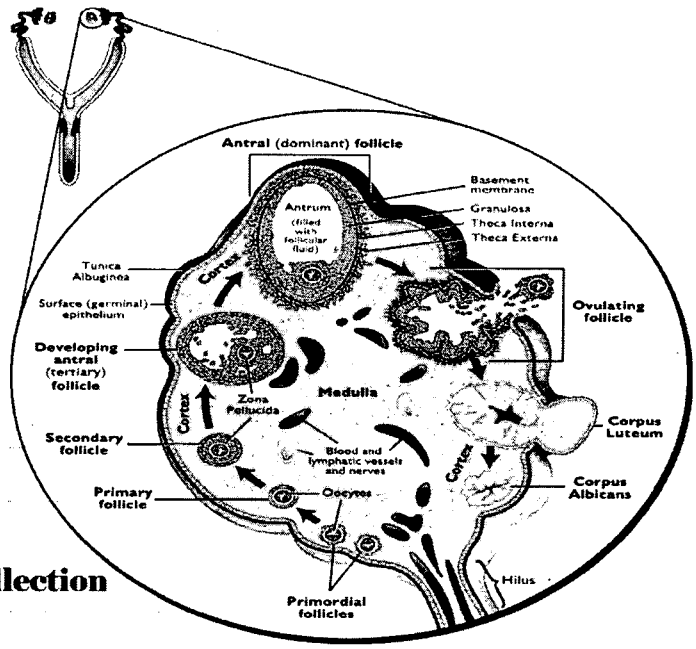
▶General Experimental Scheme

Follicle culture

Follicle growth

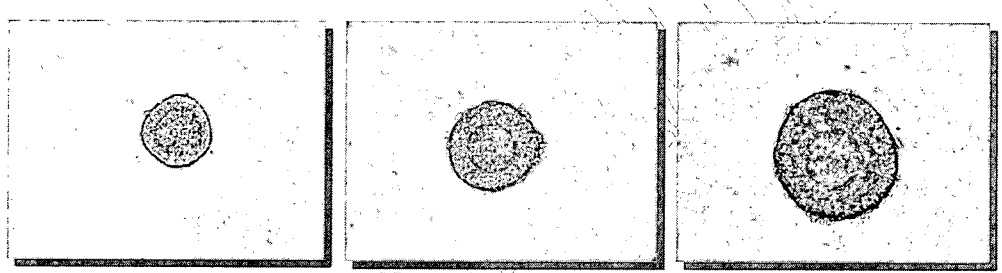
Oocyte Maturation

Collection



▶Results

Three types of preantral follicles were collected from the ovaries of sexually-immature F1 (C57BL/DBA) mice.

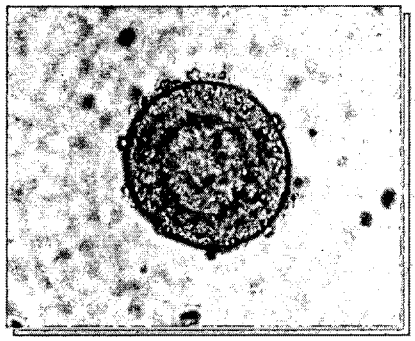


Primary **Early Secondary** **Late Secondary**

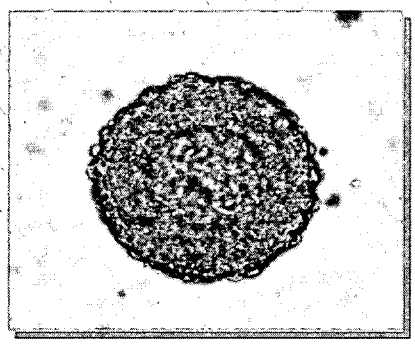
▶Lee et al. Fertil Steril (submitted)

▶ Results

Mechanical vs. Enzymatic Method: Retrieval method influenced the morphology of preantral follicles.



Mechanical

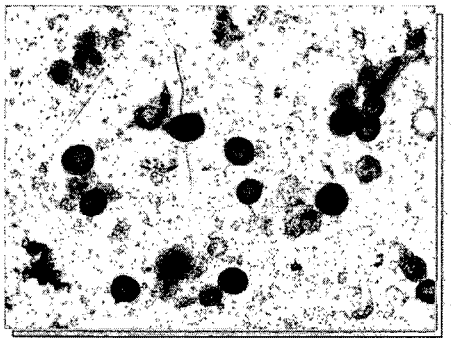


Enzymatic

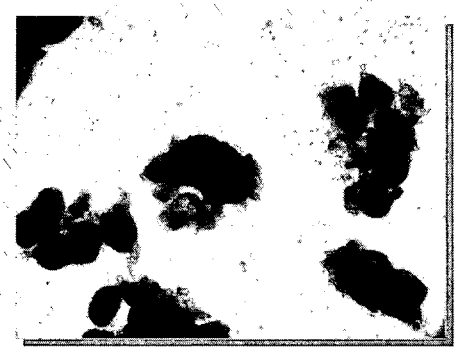
▶ Lee et al. Fertil Steril (submitted)

▶ Results

Regardless of retrieval methods, preantral follicles were retrieved singly or in groups.



Singly



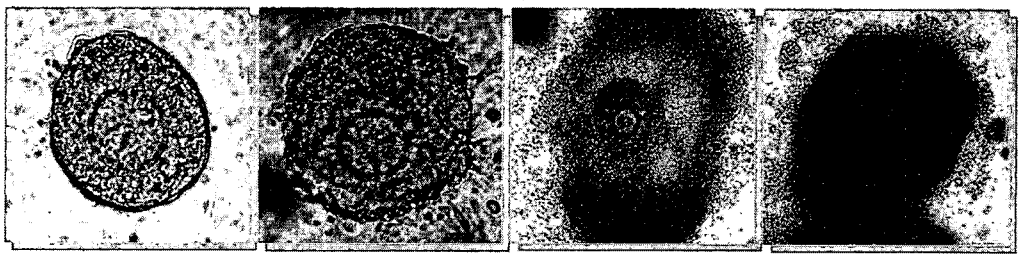
In groups

▶ Lee et al. Fertil Steril (submitted)

▶Results

When the retrieved follicles were cultured in vitro, a step-by-step growth was detected.

1. Follicular (intact) stage
2. Diffuse stage
3. Pseudoantral stage
4. Degenerative stage

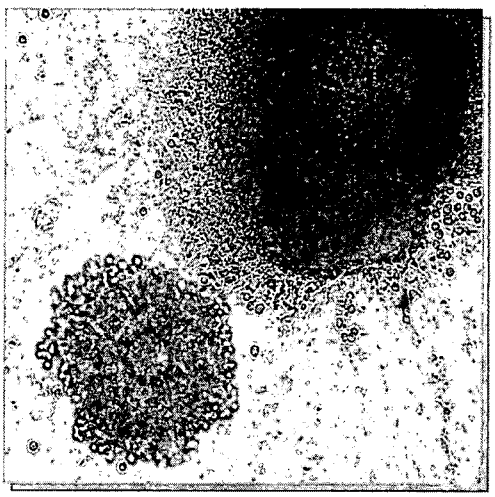


Follicular (intact) Diffuse Pseudoantral Degenerative

▶Lee et al. Fertil Steril (submitted)

▶Results

Oocyte that was ready for final maturation was released from the follicle developed to the pseudoantral stage.



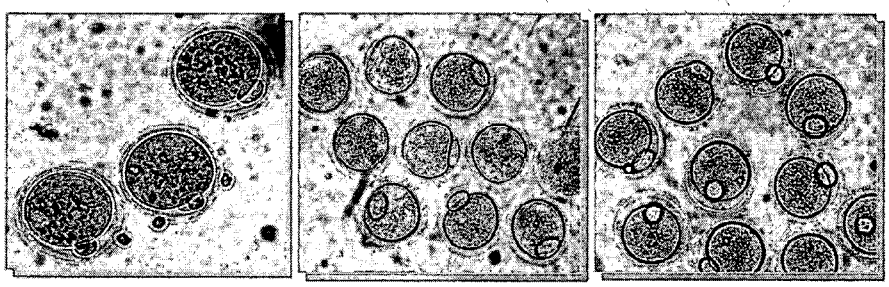
▶Lee et al. Fertil Steril (submitted)

RESULT 1:

Both the developmental stage of preantral follicle and retrieval method significantly influenced in vitro-growth of the follicle.

▶ Results

Retrieval method significantly affected morphology of mature oocytes derived from in vitro-cultured preantral follicles.



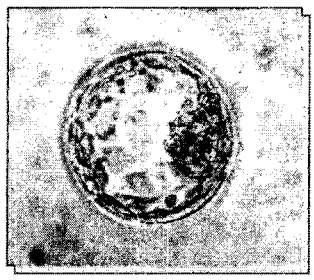
Control (in vivo matured)	Mechanical	Enzymatic
	Decreased in size	Decreased in size Reduced zona thickness

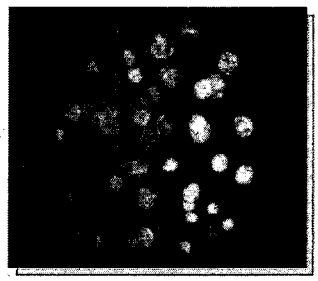
▶ Lee et al. Fertil Steril (submitted)

RESULT 2:

Both the developmental stage of preantral follicle and retrieval method affected maturation of oocytes retrieved from in vitro-cultured preantral follicle.

Blastocyst formation



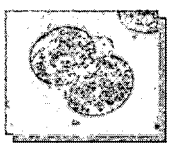


TETRAPLOID EMBRYO

▶Tetraploid

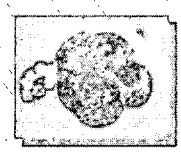
Aggregation of dipoid cells having innate or acquired pluripotency with tetraploid embryos yields great advantages in developing novel biotechnology in terms of

1) Technical feasibility
2) Good embryo development
3) Excellent model

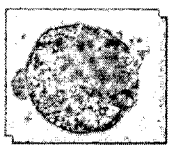


2-cell

Fusion
= Tetraploid production



Cell aggregation
(4-8-cell stage)



Morula

Blastocyst

ET > Clone live offspring
+
Culture > ES cell

▶Tetraploid

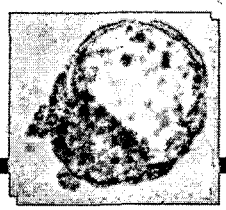
FISRT ATTEMPT:
We employed diploid cells having innate pluripotency (ES cells) as the donor cell.

Evaluated whether tetraploid embryos aggregated with ES cells could develop into normal live offsprings or transform into ES cells?

▶Ongoing Research

ET

E14 ES cell-derived clone mice



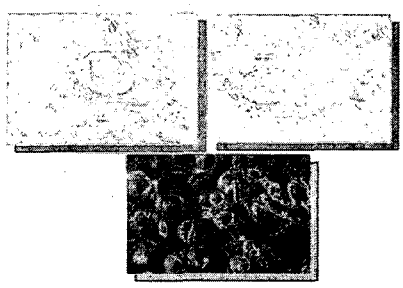
Tetraploid-E14 blastcysts derived ES cells

Culture

TERI

Body length 8cm
Tail length 6.5cm
Body weight 14g

(3 live youngs from 13 transfer)



Passage 10

(2 ES cell lines from 8 platings)

▶Cho et al. Keystone symposium (2003)

▶ Three clone live offspring from 3 pregnancies

TERI is now 2-years-old.

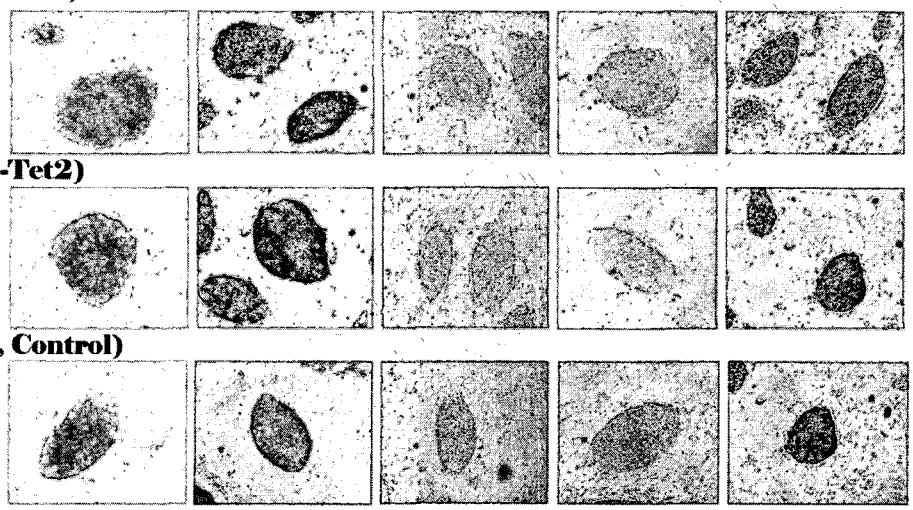
(At birth)
Body length 8cm
Tail length 6.5cm
Body weight 14g

▶ Six ES Cell lines derived from E14-aggregated tetraploid embryos

(SNUES-Tet1)

(SNUES-Tet2)

(E14 ES, Control)

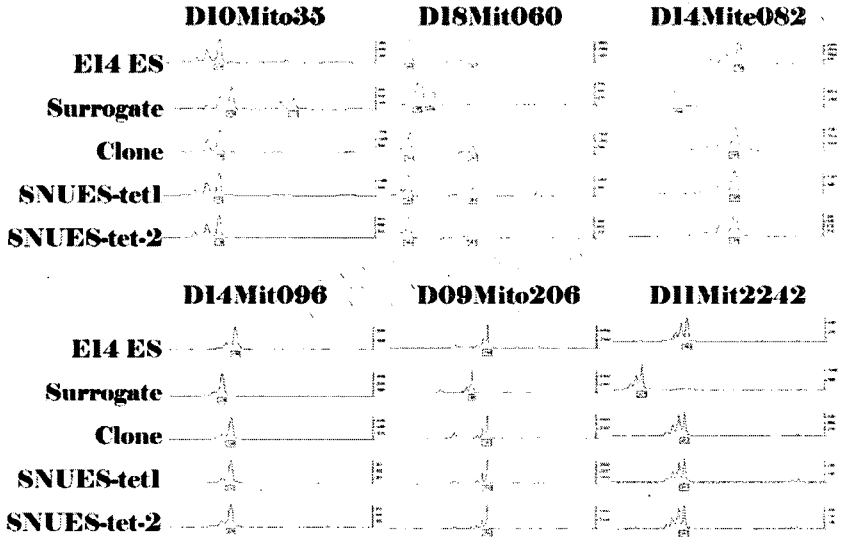


AP | Anti-SSEA-1 | Anti-SSEA-3 | Anti-SSEA-4 | Oct-4

▶ Cho et al. Keystone symposium (2005)

▶Tetraploid

Microsatellite DNA Analysis



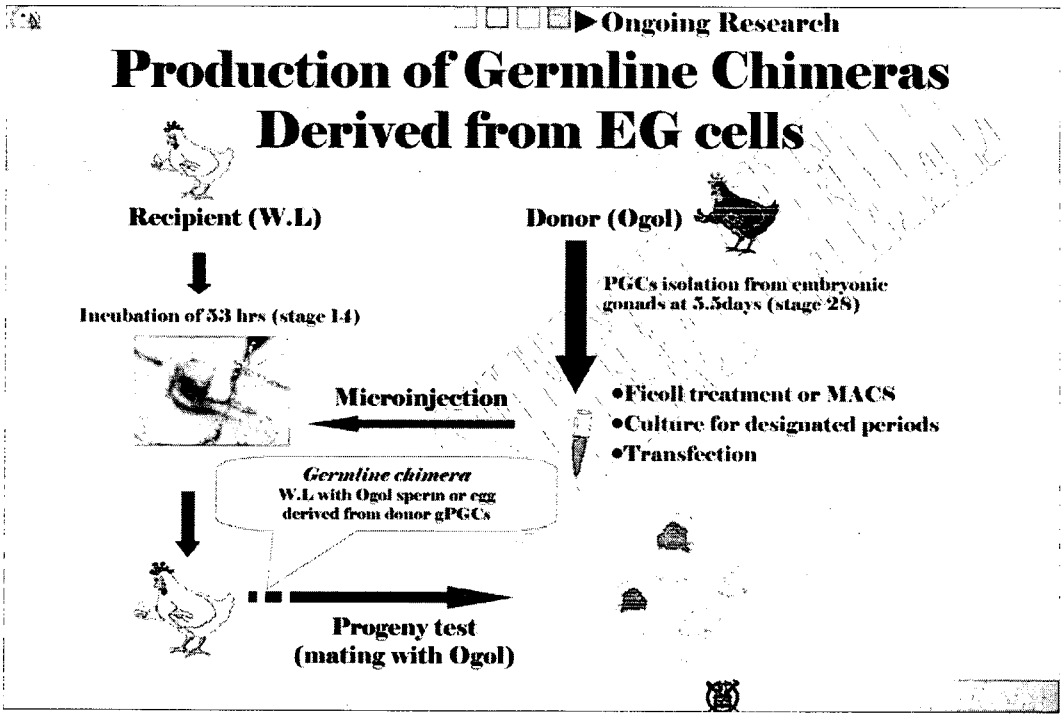
▶Cho et al. Keystone symposium (2005)

CHICKEN STEM CELL

GIFTS FROM THE BIRDS FOR BIOREACTOR PRODUCTION

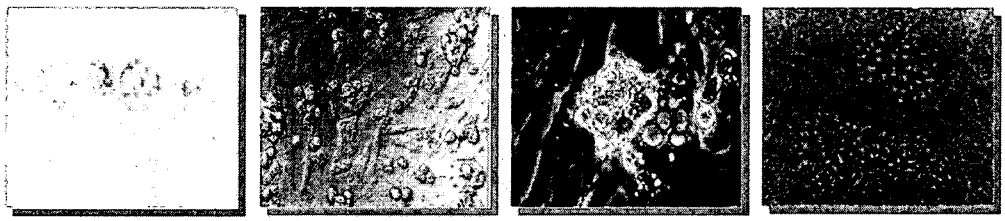
- Highly purificatory of egg protein due to simple egg composition
- Low cross species reactivity by human protein
- Mass production of specific proteins
- Being able to produce germline chimera by transferring stem cells
- Handy, appropriate size
- Short generation, highly reproductive
- Less prevalence of zoonosis
- Simple genome structure compared with mammalian species
- Genetic merit

- Germline chimeric birds
- Transgenic birds
- Model development
- Bioreactor for pharmaceutical usage
- Conserving endangered birds



EG cells can be derived by subculture of PGCs

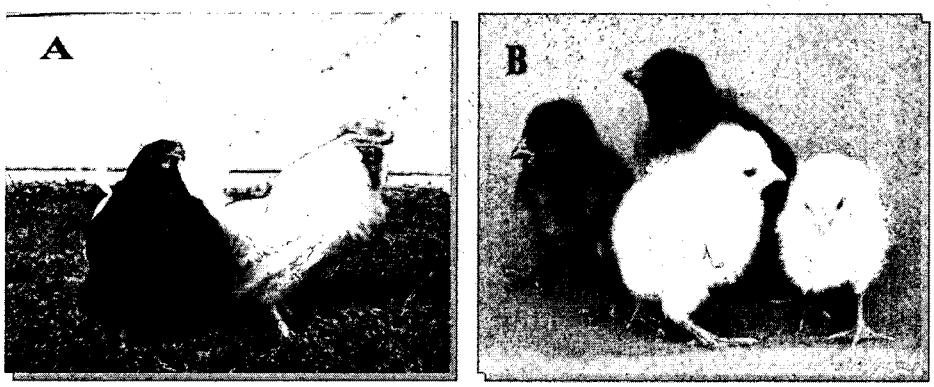
- Embryonic gonad (stage 28 to 29: 5-6 days)



Immediately after collection | On day 7 of culture | At the end of primary culture | After the third passage

(Park and Han, Mol Reprod Dev 2000)

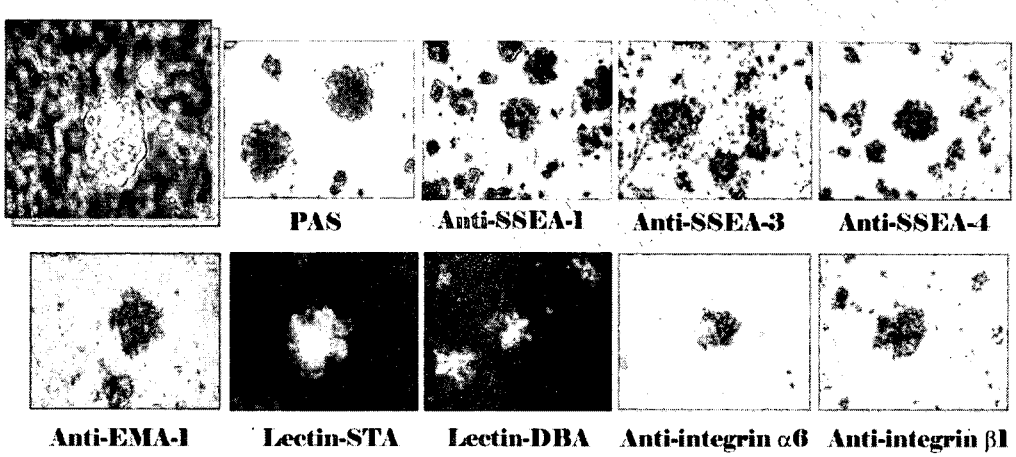
(A) White Leghorn produced by transfer of PGCs
(B) Hatched progenies



(Park et al., Mol Reprod Dev 2003)

▶Ongoing Research

Characterization of Chicken EG Cell

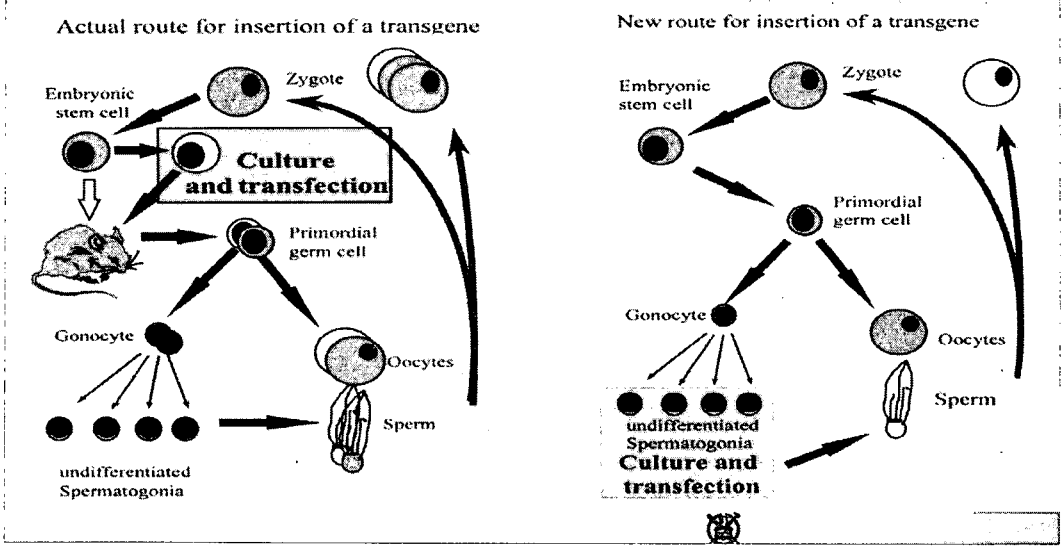


► Jung et al., Stem Cell (in press)

▶Ongoing Research

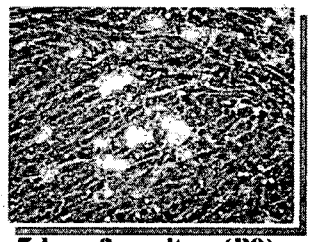
SPERMATOGONIUM;

Alternative Source of Chicken ES Cells

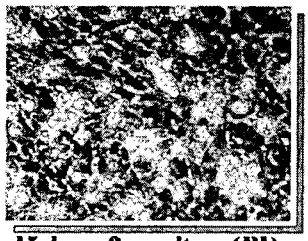


Maintenance of Spermatogonial Cells

3 days after culture (P0)



7 days after culture (P0)



15 days after culture (P1)

3 months after culture (P10)

Bioinformatics (6 persons)

-. MS coursework: 3
-. Researher: 3

Animal Molecular Genetics (20 persons)

-. Post-Doc: 1
-. PhD coursework: 10
-. MS coursework: 9

AVICORE Biotech Institute (9 persons)

-. Director: 1
-. Senior Researcher: 2 (PhD)
-. Researcher: 3 (MS)
-. Res Associate: 3 (B.Sc)

Embryology and Gamete Biotechnology (17 persons)

-. Eun J. Lee, PhD (post-doc)
-. Yong S. Lee, MS (PhD candidate)
-. Jong E. Ihm (PhD candidate)
-. Tae M. Kim (PhD candidate)
-. Sang H. Park, MS (PhD candidate)
-. Sung T. Lee (PhD candidate)
-. Mi Y. Cho (PhD coursework)
-. Ji Y. Lee (MS coursework)
-. Moon W. Lee (PhD coursework)
-. Mi Jang (PhD coursework)
-. Jae H. Lee (MS coursework)
-. Hee W. Seo (MS coursework)
-. Sung P. Gong (PhD coursework)
-. Jung K. Choi (PhD coursework)
-. Kil A Kim (PhD coursework)
-. Seo J. Oh (MS coursework)
-. Se Y. Oh, DVM (Res Assistant)