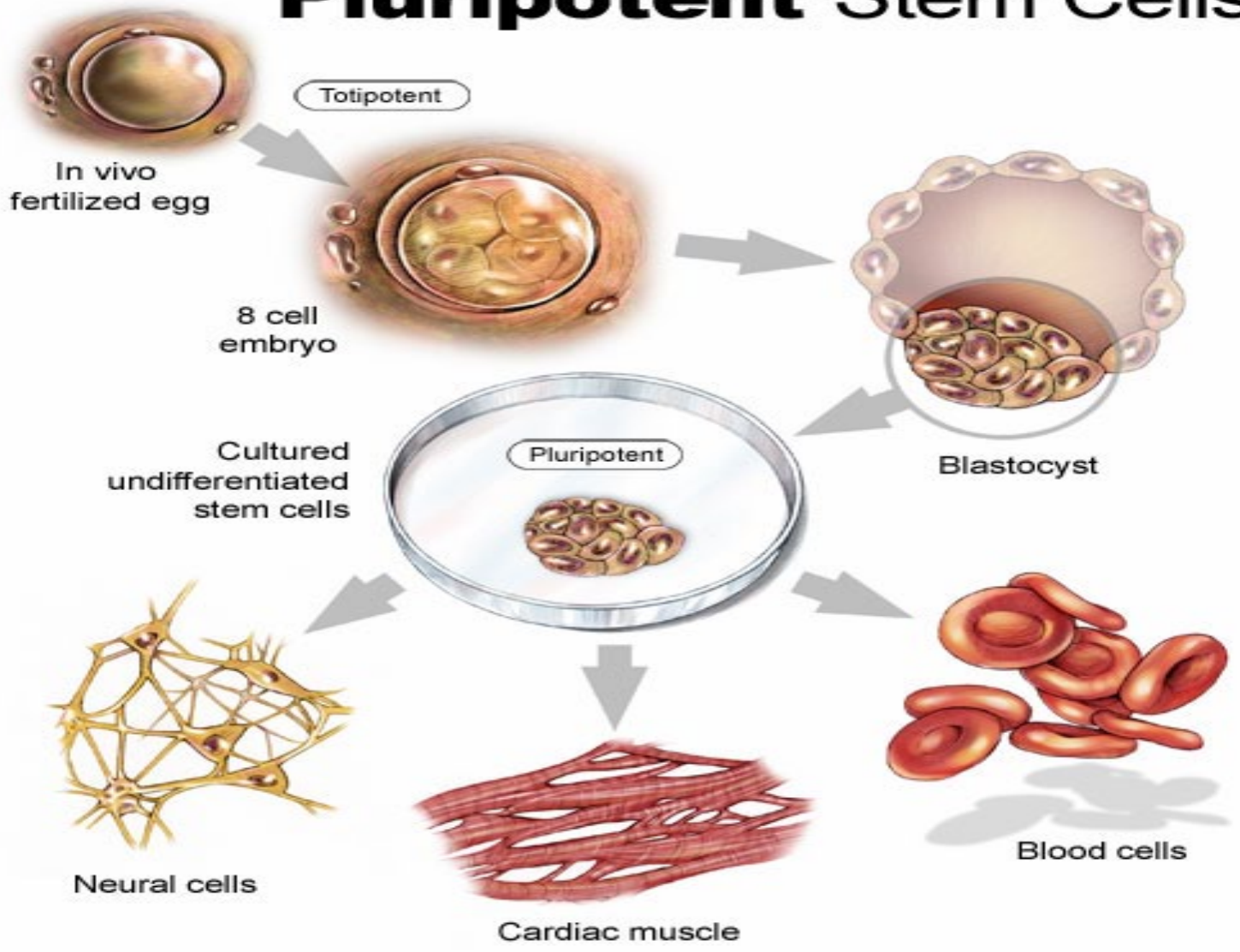
# Pluripotentne matične celice v humanem jajčniku in modu

Irma Virant-Klun

# **Pluripotent** Stem Cells



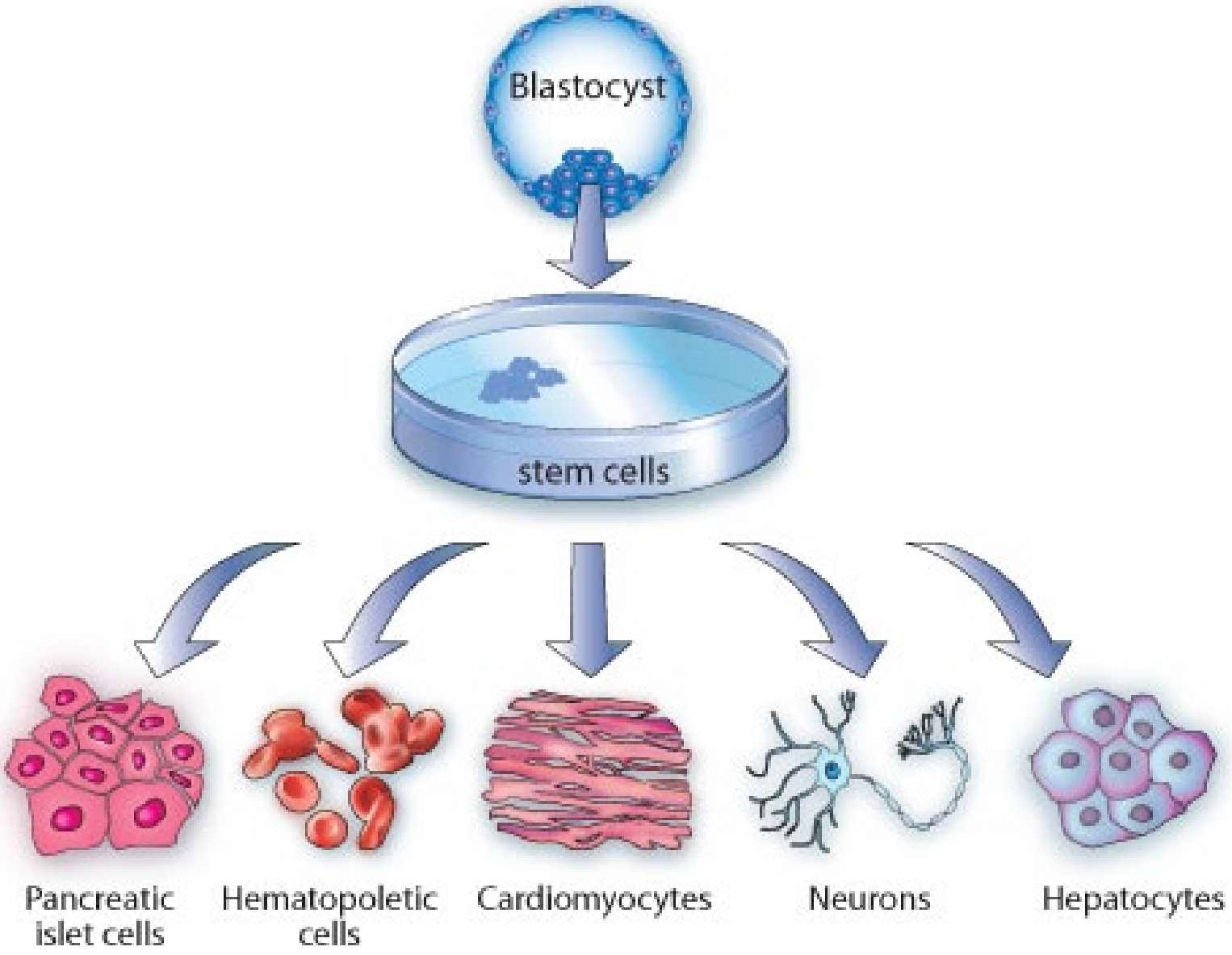
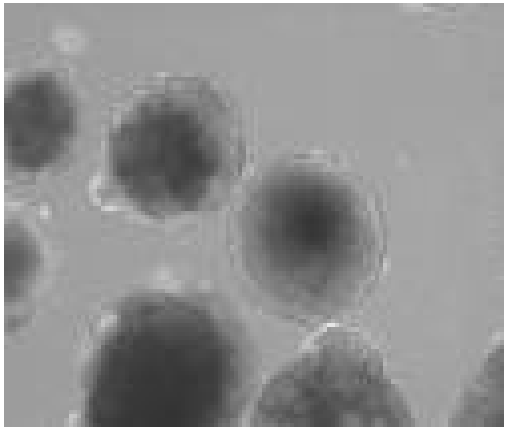
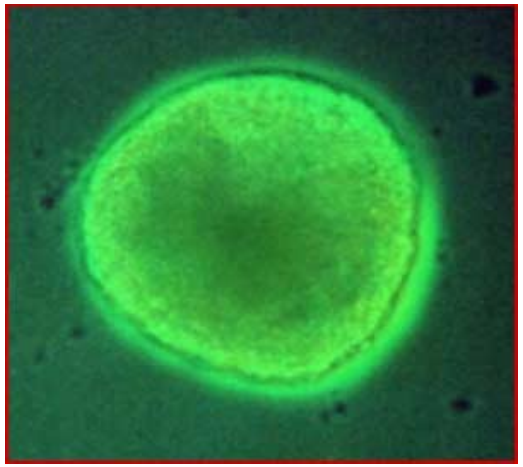



Illustration by Cell Imaging Core of the Center for Reproductive Sciences.

# Pluripotentne embrionalne matične celice

- Sposobnost neomejenega samo-obnavljanja
- Razvoj v skoraj vsak tip celic *in vivo* in *in vitro*, vključno s spolnimi celicami
- V kulturi tvorijo embriodna telesca





# Dokazovanje pluripotentnih embrionalnih matičnih celic

- Površinski antigeni (SSEA-3, SSEA-4, TRA-60, TRA-81, alkalna fosfataza) s pretočno citometrijo
- Transkripcijski markerji (Oct-4, Sox-2, Nanog, Rex-1, GAPDHACTB, DNMT3B) z RT-PCR

- Nat Biotechnol. 2007 Jul;25(7):803-16. Epub 2007 Jun 17. Links

**Characterization of human embryonic stem cell lines by the International Stem Cell Initiative.**

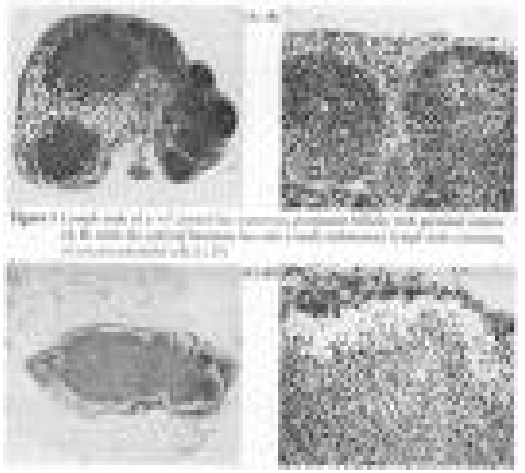
International Stem Cell Initiative, Adewumi O, Aflatoonian B, Ahrlund-Richter L, Amit M, Andrews PW, Beighton G, Bello PA, Benvenisty N, Berry LS, Bevan S, Blum B, Brooking J, Chen KG, Choo AB, Churchill GA, Corbel M, Damjanov I, Draper JS, Dvorak P, Emanuelsson K, Fleck RA, Ford A, Gertow K, Gertsenstein M, Gokhale PJ, Hamilton RS, Hampl A, Healy LE, Hovatta O, Hyllner J, Imreh MP, Itskovitz-Eldor J, Jackson J, Johnson JL, Jones M, Kee K, King BL, Knowles BB, Lako M, Lebrin F, Mallon BS, Manning D, Mayshar Y, McKay RD, Michalska AE, Mikkola M, Mileikovsky M, Minger SL, Moore HD, Mummery CL, Nagy A, Nakatsuji N, O'Brien CM, Oh SK, Olsson C, Otonkoski T, Park KY, Passier R, Patel H, Patel M, Pedersen R, Pera MF, Piekarczyk MS, Pera RA, Reubinoff BE, Robins AJ, Rossant J, Rugg-Gunn P, Schulz TC, Semb H, Sherrer ES, Siemen H, Stacey GN, Stojkovic M, Suemori H, Szatkiewicz J, Turetsky T, Tuuri T, van den Brink S, Vintersten K, Vuoristo S, Ward D, Weaver TA, Young LA, Zhang W.

UK Stem Cell Bank, Division of Cell Biology and Imaging, National Institute for Biological Standards and Control, South Mimms, Herts., EN6 3QG, UK.

The International Stem Cell Initiative characterized **59 human embryonic stem cell lines** from 17 laboratories worldwide. Despite diverse genotypes and different techniques used for derivation and maintenance, all lines exhibited similar expression patterns for several markers of human embryonic stem cells. They expressed the glycolipid antigens SSEA3 and SSEA4, the keratan sulfate antigens TRA-1-60, TRA-1-81, GCTM2 and GCT343, and the protein antigens CD9, Thy1 (also known as CD90), tissue-nonspecific alkaline phosphatase and class 1 HLA, as well as the strongly developmentally regulated genes NANOG, POU5F1 (formerly known as OCT4), TDGF1, DNMT3B, GABRB3 and GDF3. Nevertheless, the lines were not identical: differences in expression of several lineage markers were evident, and several imprinted genes showed generally similar allele-specific expression patterns, but some gene-dependent variation was observed. Also, some female lines expressed readily detectable levels of XIST whereas others did not. No significant contamination of the lines with mycoplasma, bacteria or cytopathic viruses was detected.

# Sposobnost tvorbe tumorjev

- Subkutano injiciranje celic v miško SCID/NOD z zmanjšano imunsko odpornostjo – tvorba tumorjev (teratomov)
- Histološka analiza tumorjev



# Najnovejše raziskave

- Embrionalne matične celice so prisotne tudi v odraslih tkivih in organih človeka

- **Hipoteza:**

  kot rezerva se ohranjajo iz embrionalnega obdobja življenja

# Ratajczak in Kucia s sodelavci,

Stem Cell Biology Program at James Graham Brown Cancer Center, University of Louisville, Louisville, Cancer Center, Louisville, ZDA

## Pluripotentne embrionalne matične celice v kostnem mozgu človeka.

Ratajczak MZ, Machalinski B, Wojakowski W, Ratajczak J, Kucia M.

A hypothesis for an embryonic origin of pluripotent Oct-4(+) stem cells in adult bone marrow and other tissues.Leukemia. 2007 May;21(5):860-7.

# Beltrami s sodel.,

Centro Interdipartimentale Medicina Rigenerativa, University of Udine,
Piazzale Santa Maria della Misericordia, 33100 Udine,

Pluripotentne embrionalne matične celice v različnih tkivih in organih odraslega človeka – srce, jetra in kostni mozeg.

Beltrami AP, Cesselli D, Bergamin N, Marcon P, Rigo S, Puppato E, D'Aurizio F, Verardo R, Piazza S, Pignatelli A, Poz A, Baccarani U, Damiani D, Fanin R, Mariuzzi L, Finato N, Masolini P, Burelli S, Belluzzi O, Schneider C, Beltrami CA. Multipotent cells can be generated in vitro from several adult human organs (heart, liver, and bone marrow). Blood. 2007 Nov 1;110(9):3438-46. Epub 2007 May 24.

# Pluripotentne embrionalne matične celice v odraslem testisu

- miši (Univerza Gottingen, Nemčija),

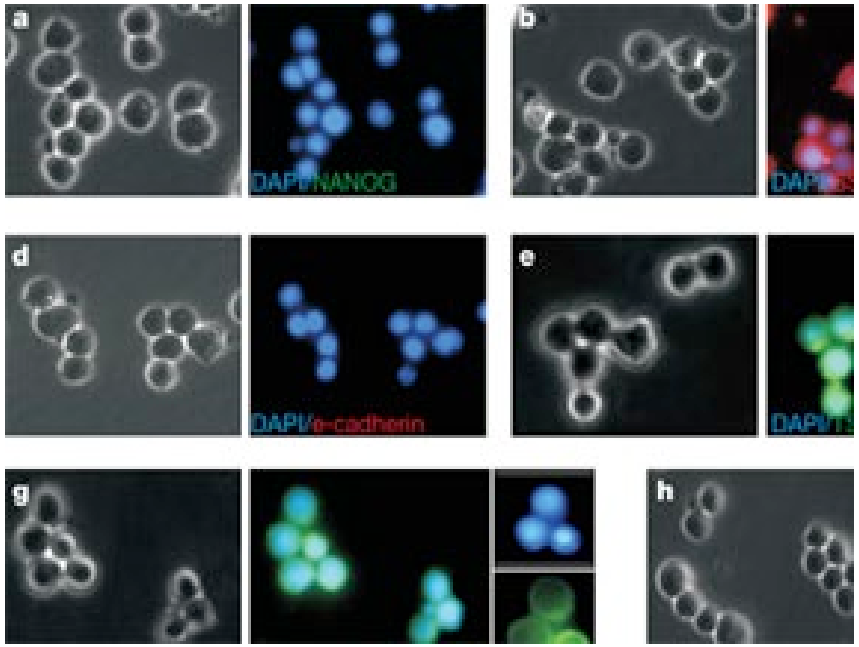
  Guan K, Nayernia K, Maier LS, Wagner S, Dressel R, Lee JH, Nolte J, Wolf F, Li M, Engel W, Hasenfuss G. Pluripotency of spermatogonial stem cells from adult mouse testis. Nature. 2006 Apr 27;440(7088):1199-203.

  Guan K, Wagner S, Unsöld B, Maier LS, Kaiser D, Hemmerlein B, Nayernia K, Engel W, Hasenfuss G.
  Generation of functional cardiomyocytes from adult mouse spermatogonial stem cells. Circ Res. 2007 Jun 8;100(11):1615-25. .

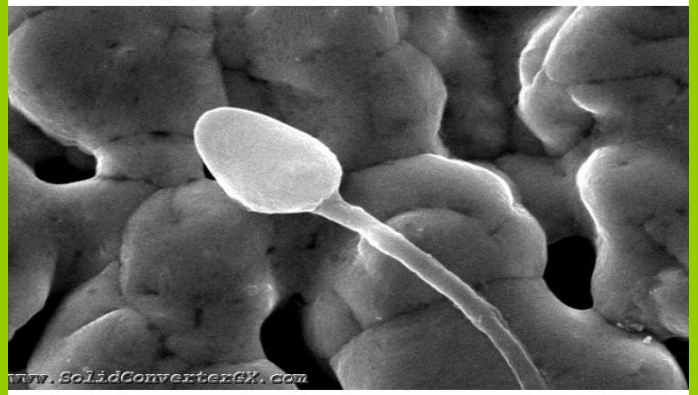
- človeka (Univerza Tubingen, Nemčija)
  - Skutella T. : *in vitro* spermatogeneza

# **IZJEMNO:** Pluripotentne matične celice izolirane iz humanega testisa !

Generation of pluripotent stem cells from adult human testis. Conrad S, Renninger M, Hennenlotter J, Wiesner T, Just L, Bonin M, Aicher W, Bühring HJ, Mattheus U, Mack A, Wagner HJ, Minger S, Matzkies M, Reppel M, Hescheler J, Sievert KD, Stenzl A, Skutella T. *Nature 2008 Nov 20;456(7220):344-9.*




# Razvoj embrionalnih matičnih celic v spolne celice



# Razvoj pluripotetnih mišjih embrionalnih matičnih celic v jajčne celice

Science 2003 May 23;300(5623):1251-6. Epub 2003 May 1. Links

- **Derivation of oocytes from mouse embryonic stem cells.**

Hübner K, Fuhrmann G, Christenson LK, Kehler J, Reinbold R, De La Fuente R, Wood J, Strauss JF 3rd, Boiani M, Schöler HR.

Germline Development Group, Center for Animal Transgenesis and Germ Cell Research, School of Veterinary Medicine, University of Pennsylvania, New Bolton Center, 382 West Street Road, Kennett Square, PA 19348, USA.

Continuation of mammalian species requires the formation and development of the sexually dimorphic germ cells. Cultured embryonic stem cells are generally considered pluripotent rather than totipotent because of the failure to detect germline cells under differentiating conditions. Here we show that **mouse embryonic stem cells in culture can develop into oogonia that enter meiosis, recruit adjacent cells to form follicle-like structures, and later develop into blastocysts.** Oogenesis in culture should contribute to various areas, including nuclear transfer and manipulation of the germ line, and advance studies on fertility treatment and germ and somatic cell interaction and differentiation.

Stem Cells. 2006 Aug;24(8):1931-6. Epub 2006 Apr 27. Links

- **Mouse embryonic stem cells form follicle-like ovarian structures but do not progress through meiosis.**

Novak I, Lightfoot DA, Wang H, Eriksson A, Mahdy E, Höög C.
Department of Cell and Molecular Biology, Karolinska Institutet, SE-171 77, Stockholm, Sweden.

Several recent studies have suggested that mouse embryonic stem cells (ESCs) can differentiate into female and male germ cells in vitro. The meiotic process in germ cell-like cells derived from ESCs has not been studied in detail, but it has been reported that synaptonemal complex protein-3 (SYCP3) is expressed in these cells. Here, we have carefully evaluated the meiotic process in germ cell-like cells derived from ESCs, using a panel of meiosis-specific markers that identify distinct meiotic signatures unique to meiotic prophase I development in vivo. We find that whereas SYCP3 is expressed in germ cell-like cells, other meiotic proteins, such as SYCP1, SYCP2, STAG3 (stromal antigen 3), REC8 (meiotic protein similar to the rad21 cohesins), and SMC1 (structural maintenance of chromosomes-1)-beta, are not expressed. The nuclear distribution of SYCP3 in the germ cell-like cells is highly abnormal and not associated with the chromosomes of these cells. Fluorescence in situ hybridization analysis shows that the SYCP3-positive germ cell-like cells do not contain synapsed homologous chromosomes but instead display a chromosomal organization normally found in somatic cells. The absence of expression of essential meiotic proteins and a normal meiotic chromosomal organization strongly suggests that the germ cell-like cells formed from ESCs fail to progress through meiosis.

- Stem Cells. 2006 Feb;24(2):266-73. Epub 2005 Aug 18. Links

  - **Testicular cell conditioned medium supports differentiation of embryonic stem cells into ovarian structures containing oocytes.**

    Lacham-Kaplan O, Chy H, Trounson A.

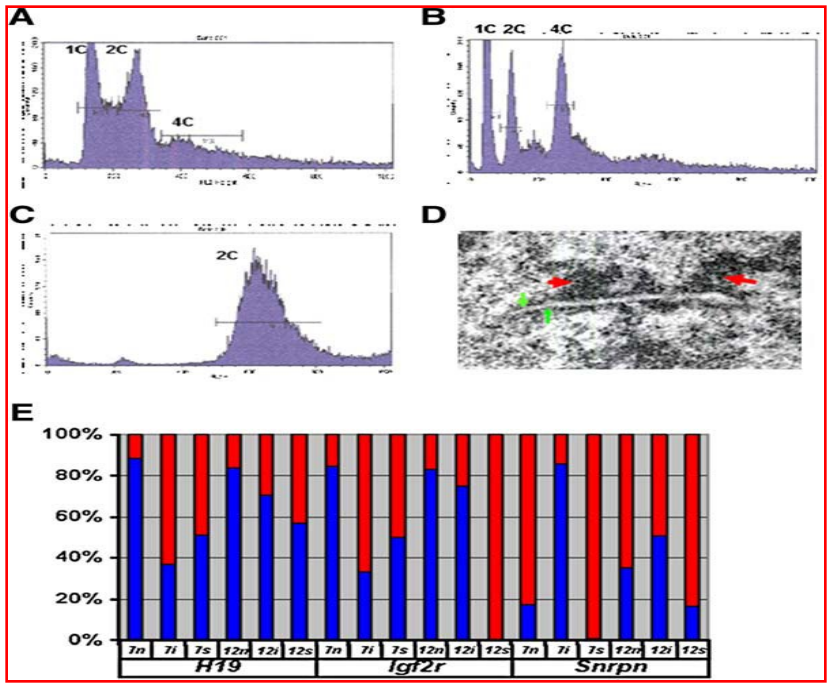
    Monash Immunology and Stem Cell Laboratories, Monash University, Wellington Rd., Clayton, Australia 3165. Orly.Lacham-kaplan@med.monash.edu.au

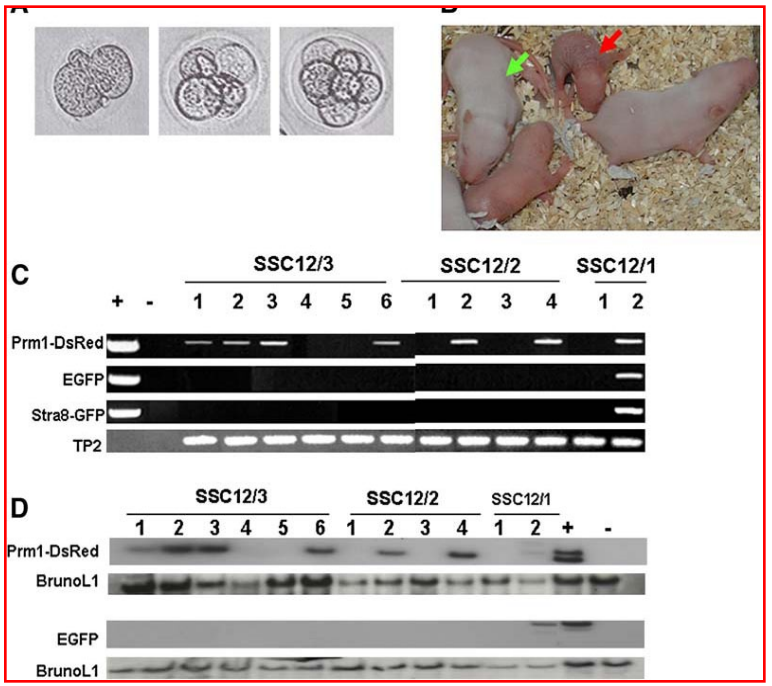
    Previous reports and the current study have found that germ cell precursor cells appear in embryoid bodies (EBs) formed from mouse embryonic stem cells as identified by positive expression of specific germ cell markers such as Oct-3/4, Mvh, c-kit, Stella, and DAZL. We hypothesized that if exposed to appropriate growth factors, the germ cell precursor cells within the EBs would differentiate into gametes. The source for growth factors used in the present study is conditioned medium collected from testicular cell cultures prepared from the testes of newborn males. Testes at this stage of development contain most growth factors required for the transformation of germ stem cells into differentiated gametes. When EBs were cultured in the conditioned medium, they developed into ovarian structures, which contained putative oocytes. The oocytes were surrounded by one to two layers of flattened cells and did not have a visible zona pellucida. However, oocyte-specific markers such as Fig-alpha and ZP3 were found expressed by the ovarian structures. The production of oocytes using this method is repeatable and reliable and may be applicable to other mammalian species, including the human.

# Spermatogeneza *in vitro*

**In Vitro-Differentiated Embryonic Stem Cells Give Rise to Male Gametes that Can Generate Offspring Mice.** Nayernia H, Nolte J, Michelmann HW, Lee JH, Rathsack K, Drusenheimer N, Dev A, Wulf G, Ehrmann IE, Elliott DJ, Okpanyi V, Zechner U, Haaf T, Meinhardt A, and Engel W.

*Developmental Cell 11, 125–132, July, 2006*



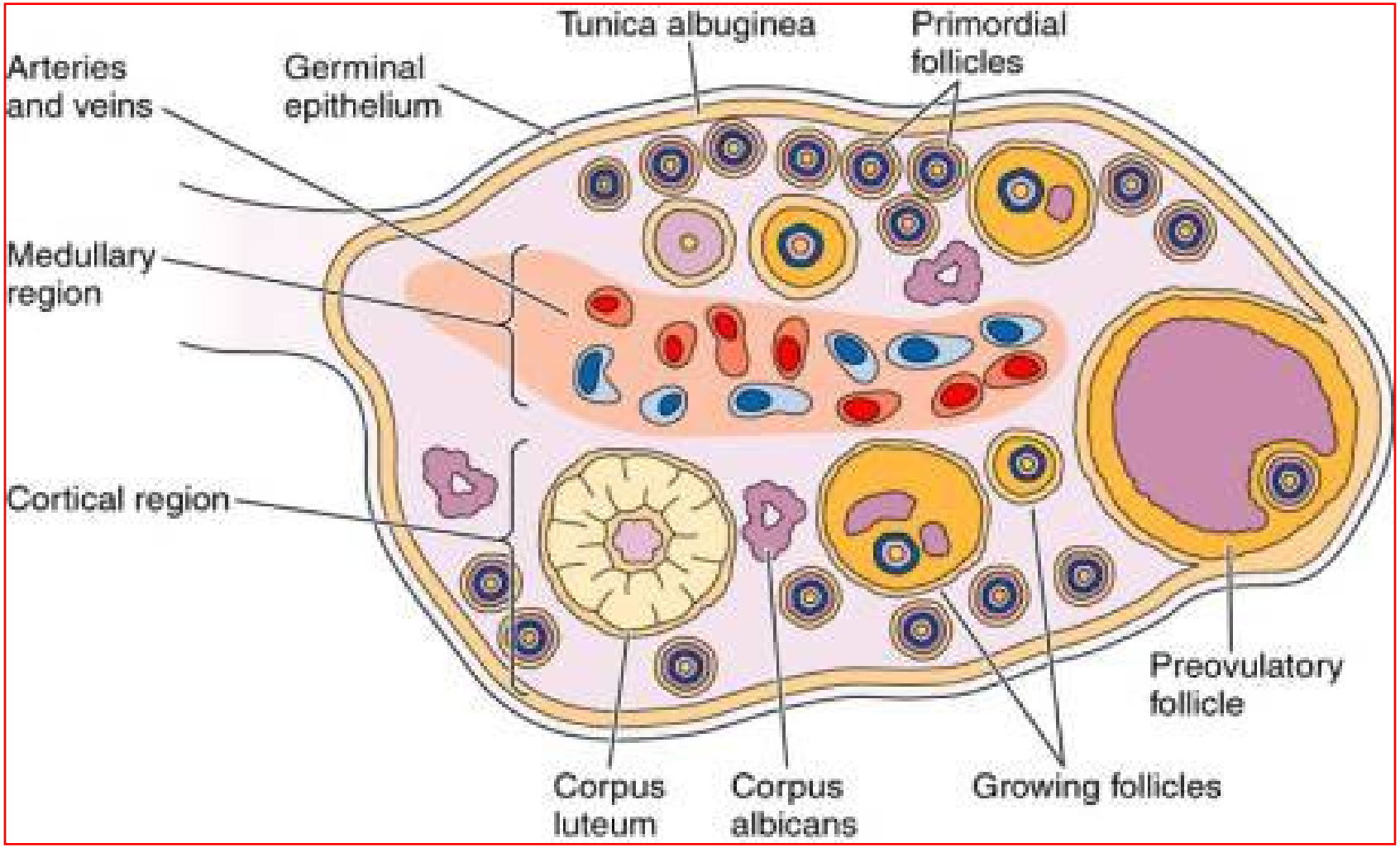



# Razvoj pluripotetnih humanih embrionalnih matičnih iz kostnega mozga moškega v spermije

- Soc Reprod Fertil Suppl. 2007;63:69-76.Links
  - **Putative human male germ cells from bone marrow stem cells.**
  - Drusenheimer N, Wulf G, Nolte J, Lee JH, Dev A, Dressel R, Gromoll J, Schmidtke J, Engel W, Nayernia K.
  - Institute of Human Genetics, University of Göttingen, D-37073 Göttingen.
  - Germ cells must develop along distinct male or female paths to produce the spermatozoa or oocyte required for sexual reproduction. Male germline stem cells maintain spermatogenesis in the postnatal human testis. Here we show that a small population of bone marrow cells is able to transdifferentiate to male germ cell-like cells. We show expression of **early germ cell markers (Oct4, Fragilis, Stella and Vasa)** and **male germ cell specific markers (Dazl, TSPY, Piwil2 and Stra8)** in these cells. Our preliminary findings provide direct evidence that human bone marrow cells can differentiate to putative male germ cells and identify bone marrow as a potential source of male germ cells that could sustain sperm production.
  - PMID: 17566262 [PubMed - indexed for MEDLINE]

# Predstavitev naših raziskovalnih rezultatov

# Odrasli humani jajčnik



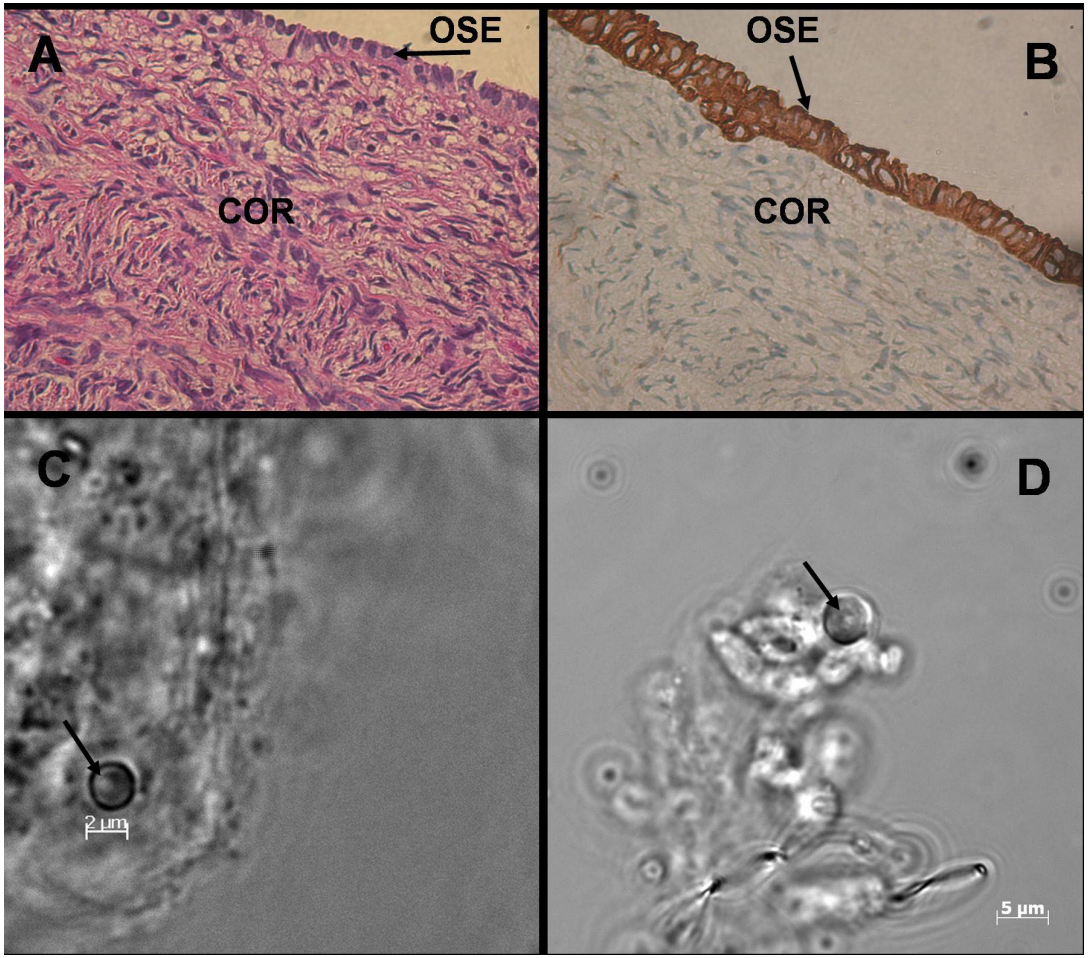
# Pluripotentne ebrionalne matične celice v odraslem jajčniku

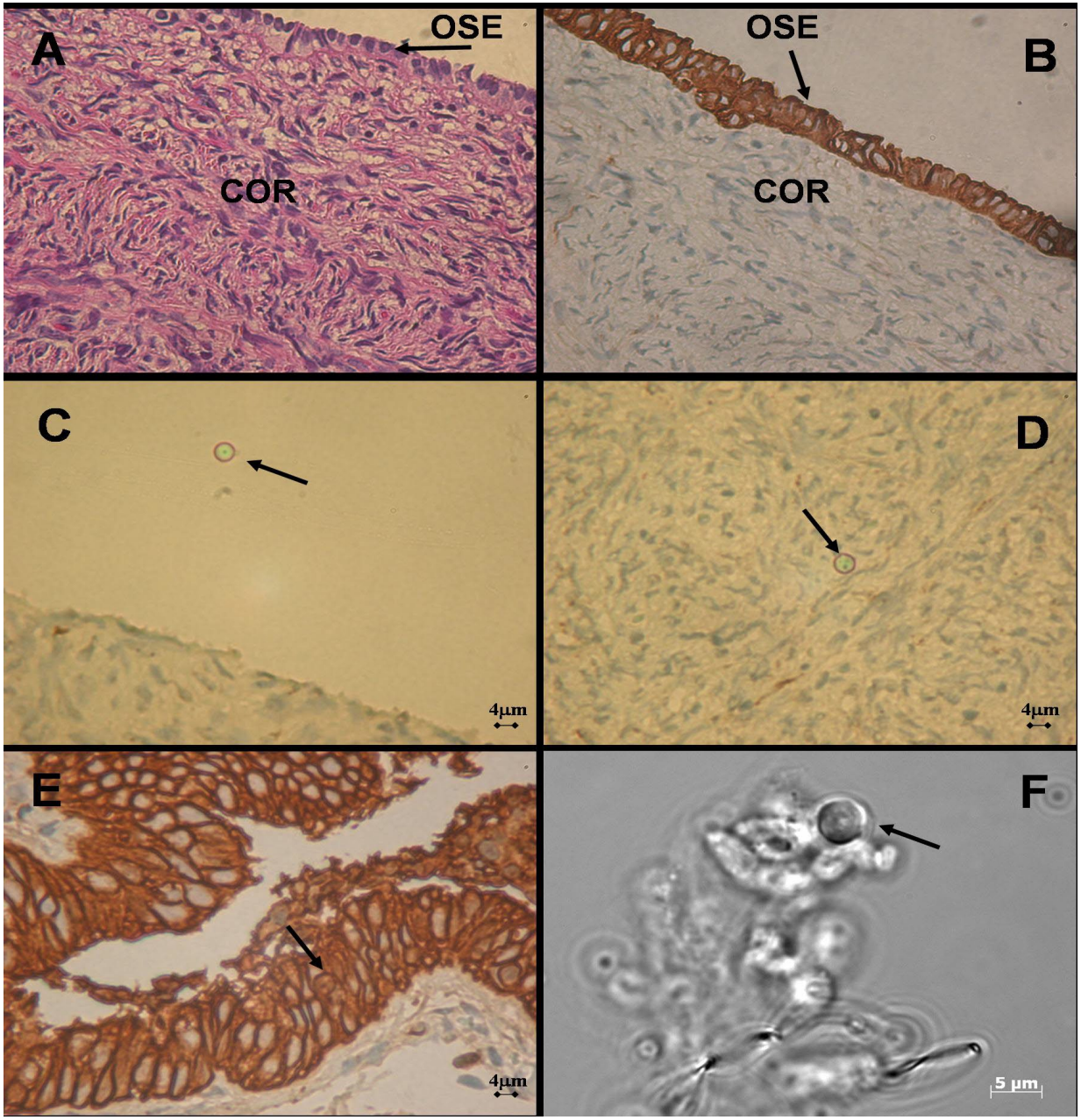
- Pomenopavzne ženske
- Mlade ženske s prezgodnjo menopavzo

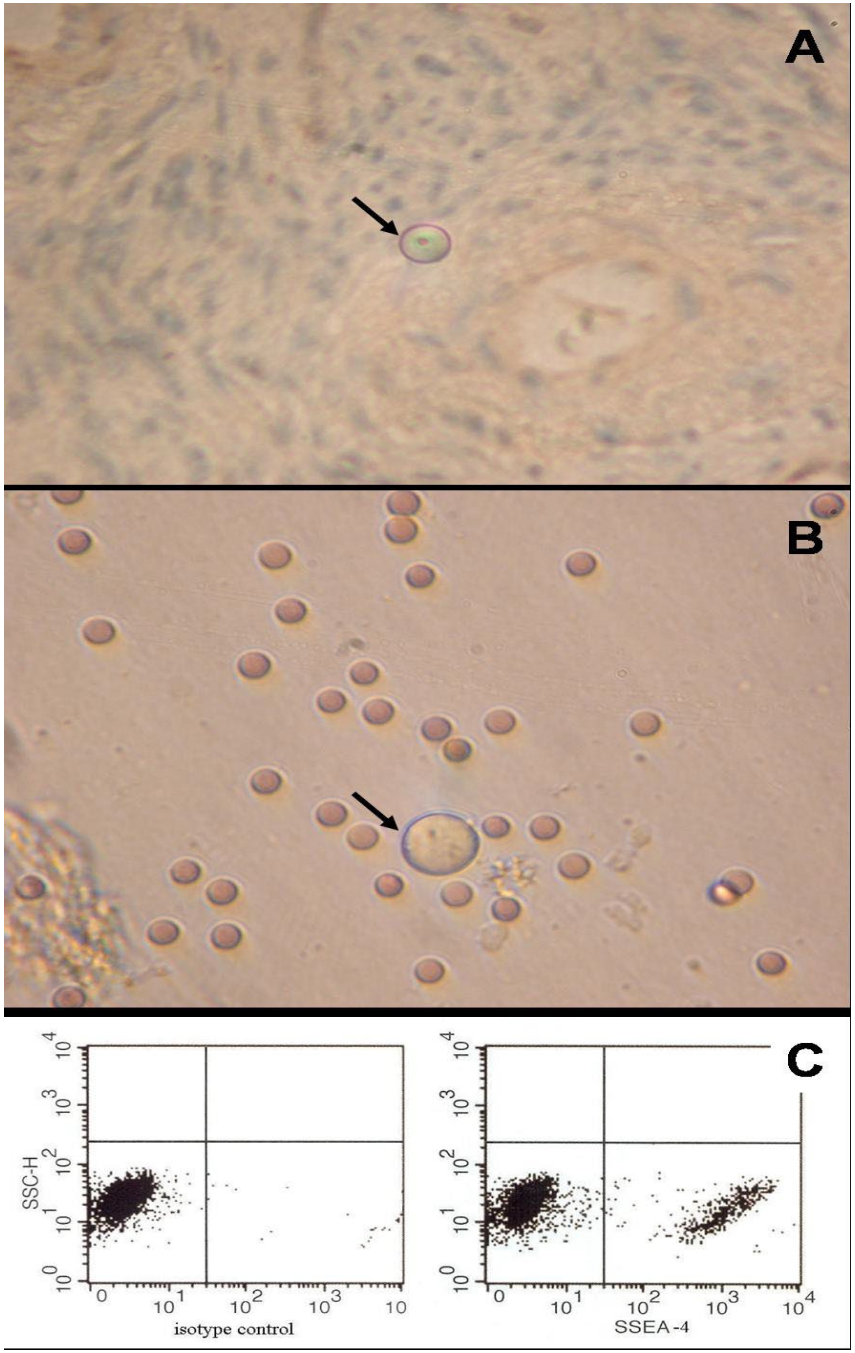
**Celična kultura površinskega epitelija jajčnika**

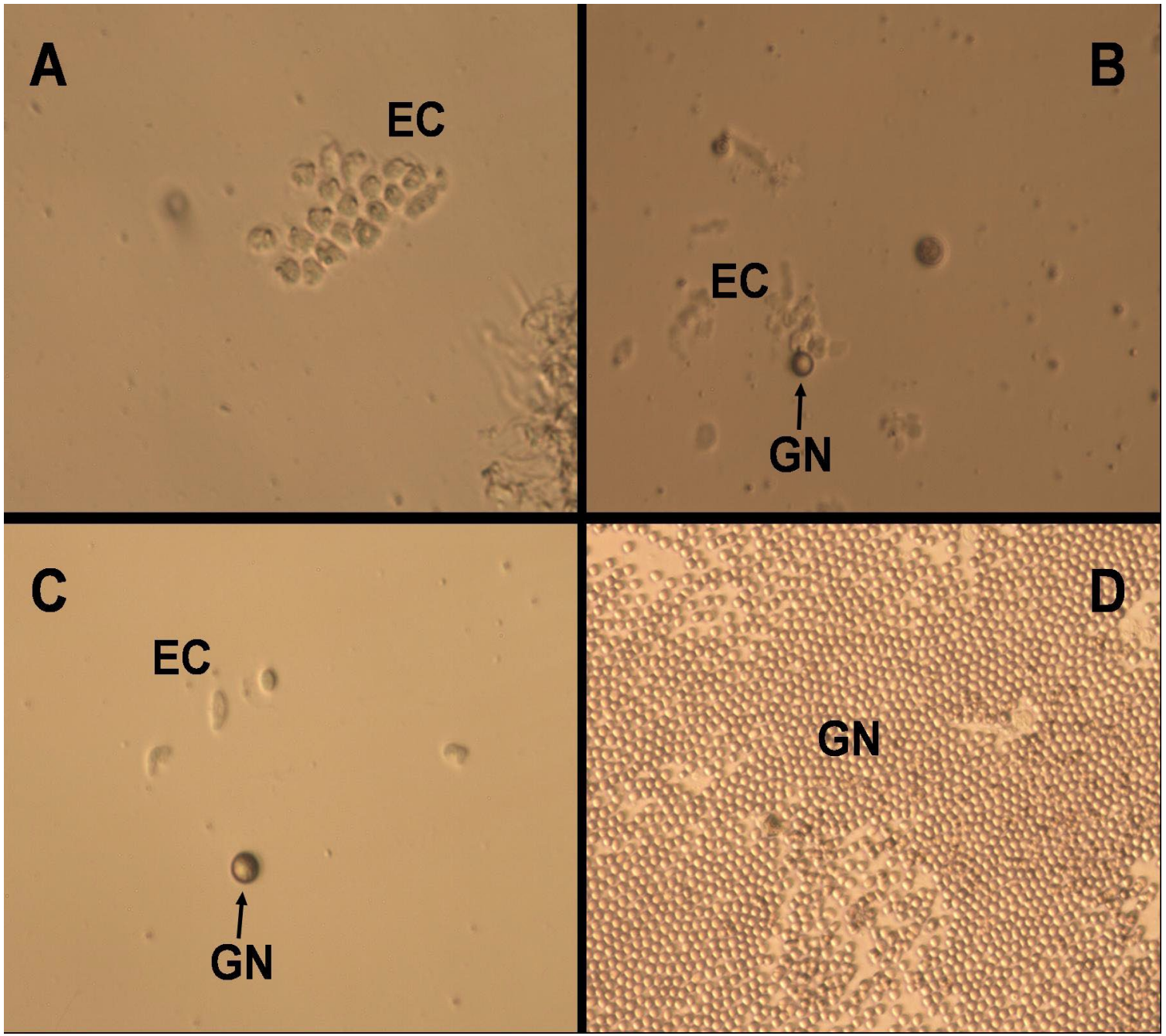
(DMEM/F-12, fenol rdeče)

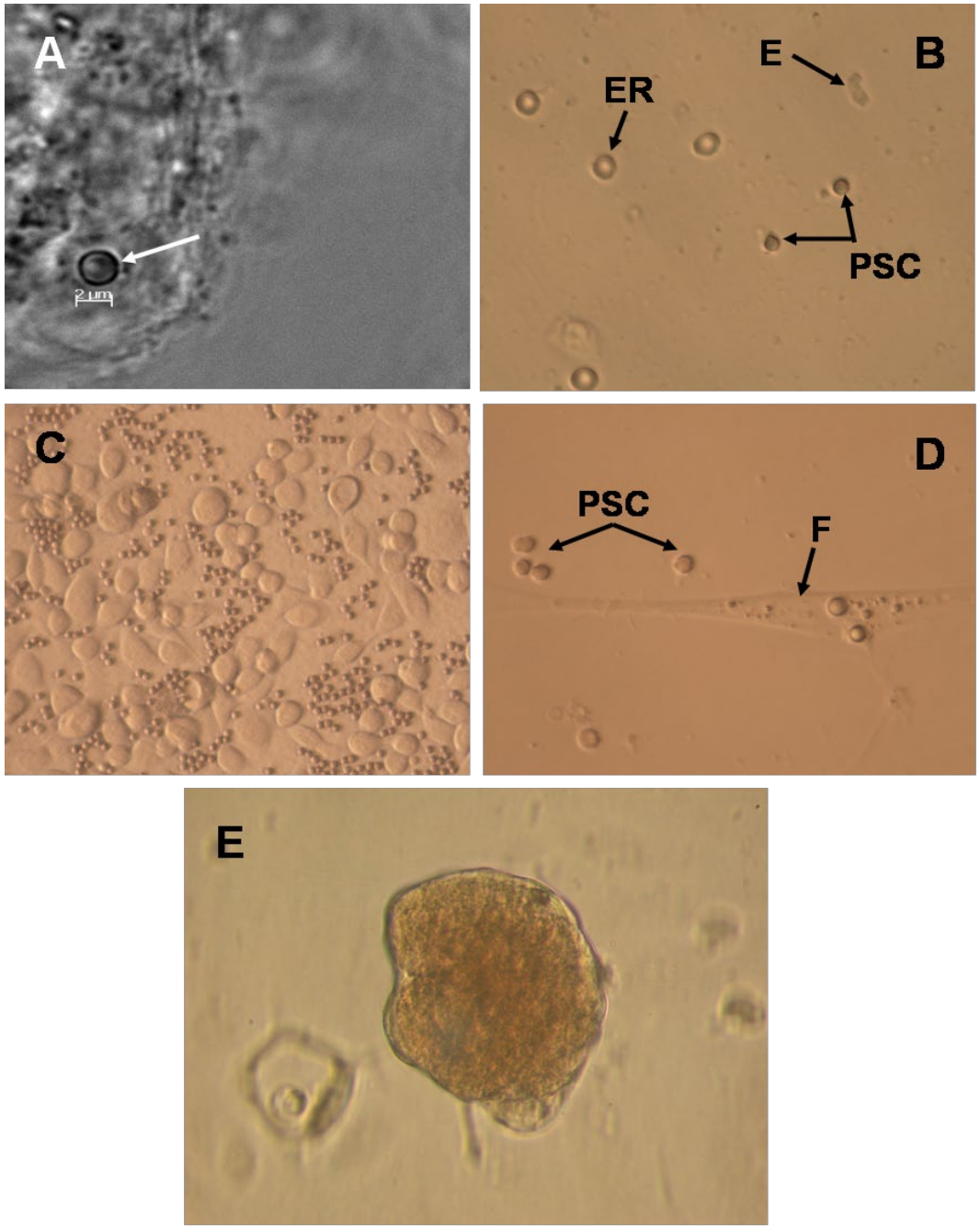
# Prisotnost do sedaj še neopisanih celic v jajčniku

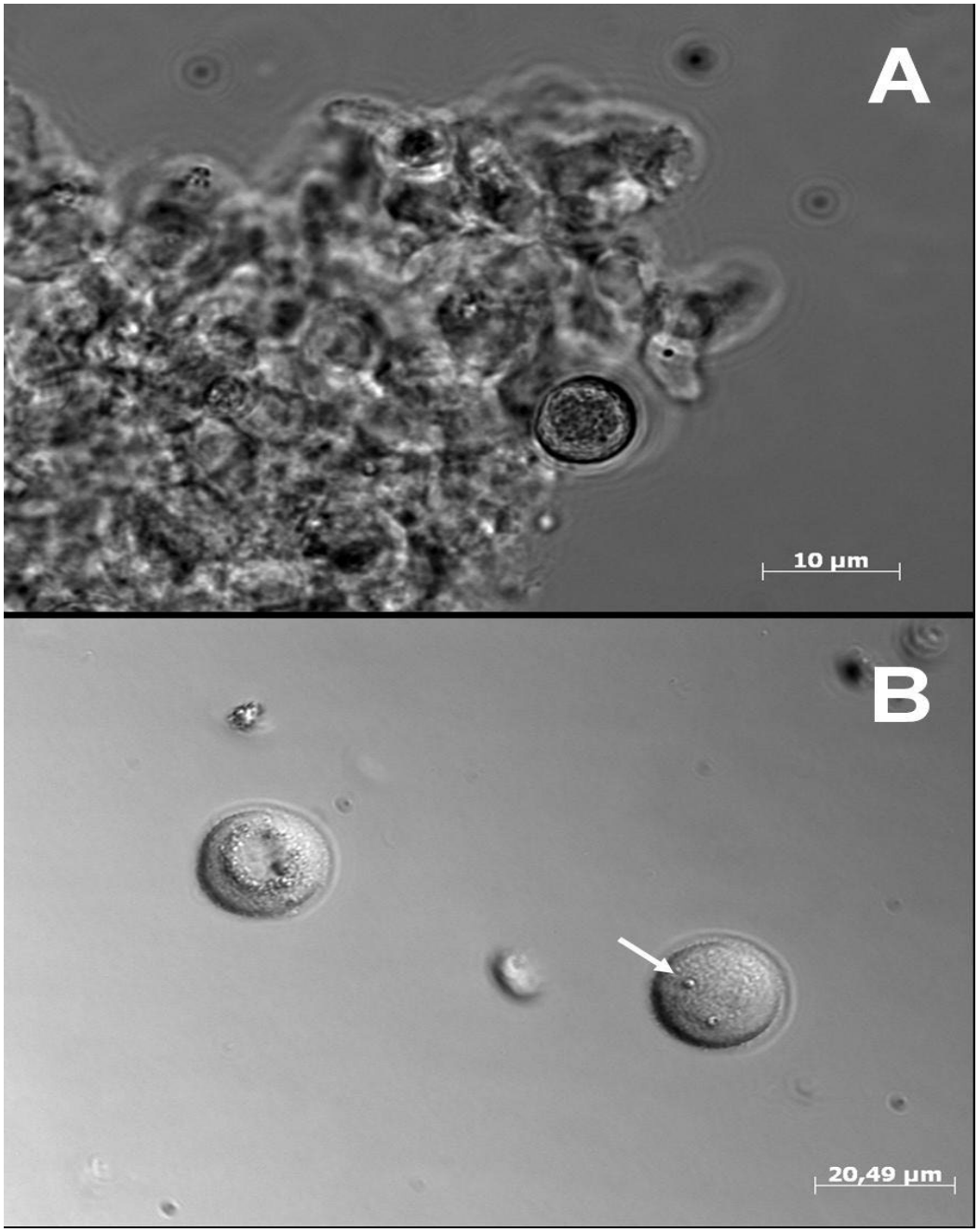


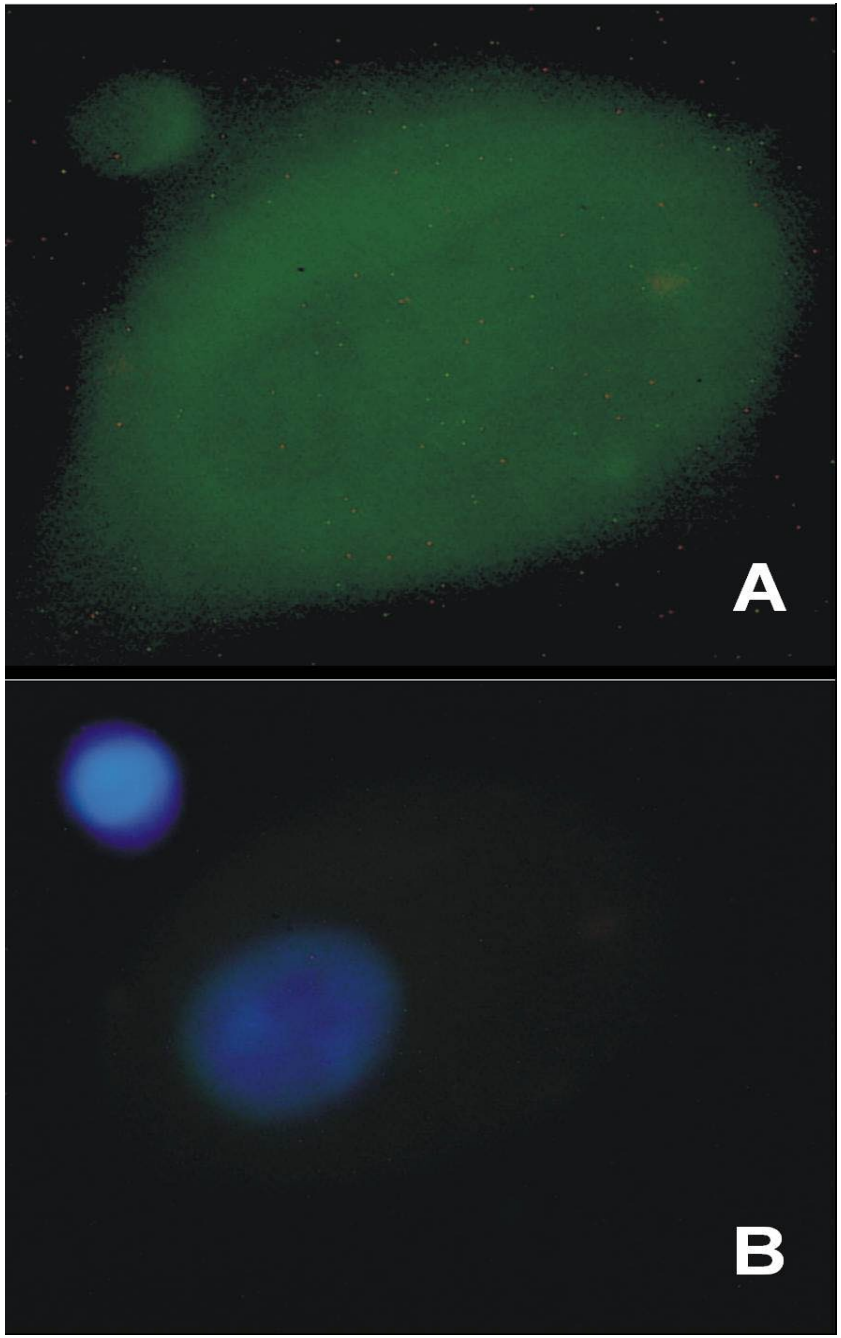

A
OSE
COR
B
OSE
COR
C
4µm
D
4µm
E
4µm
F
5 µm

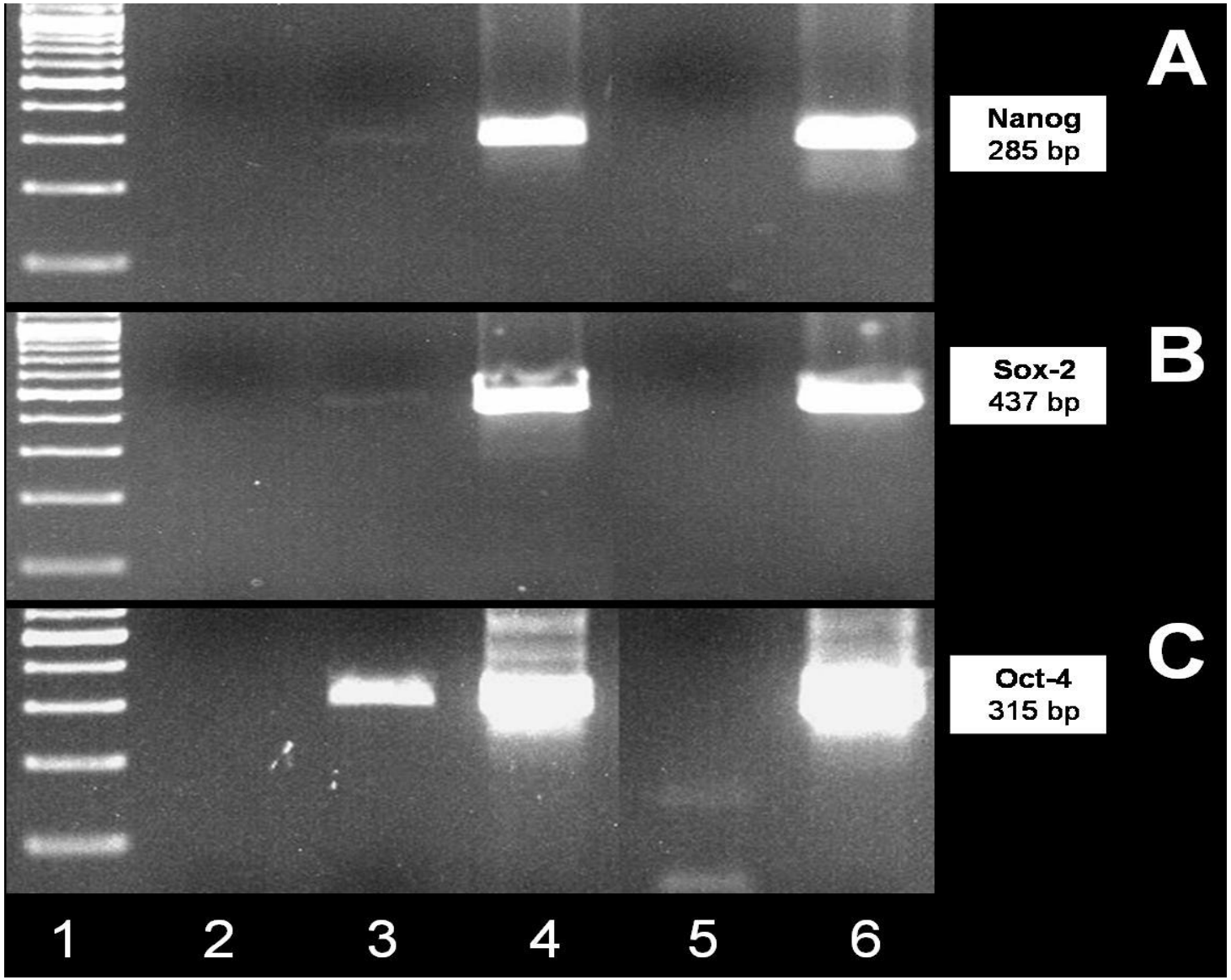

A
B
C
SSC-H
isotype control
SSEA-4

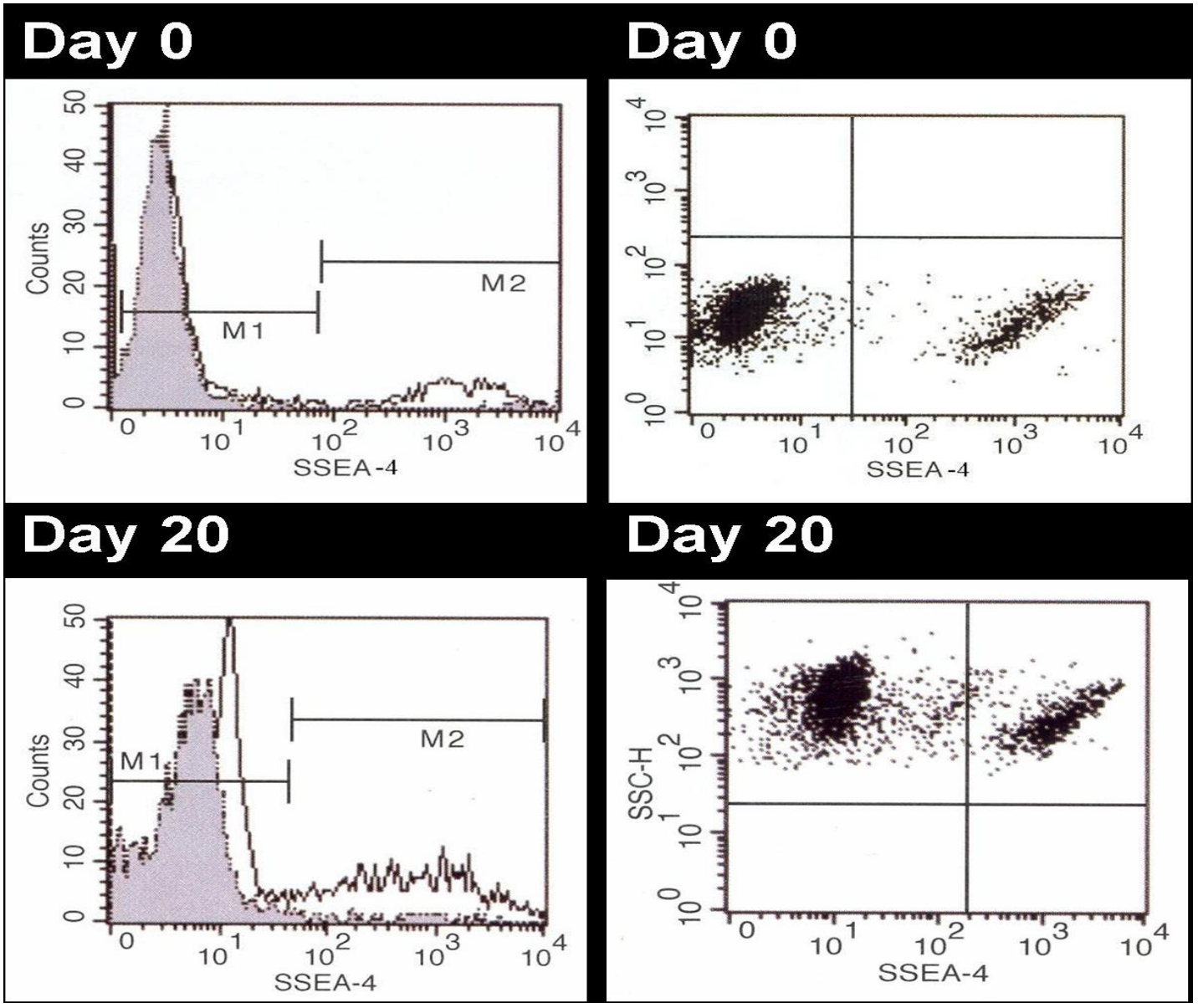

A
EC
B
EC
GN
C
EC
GN
D
GN

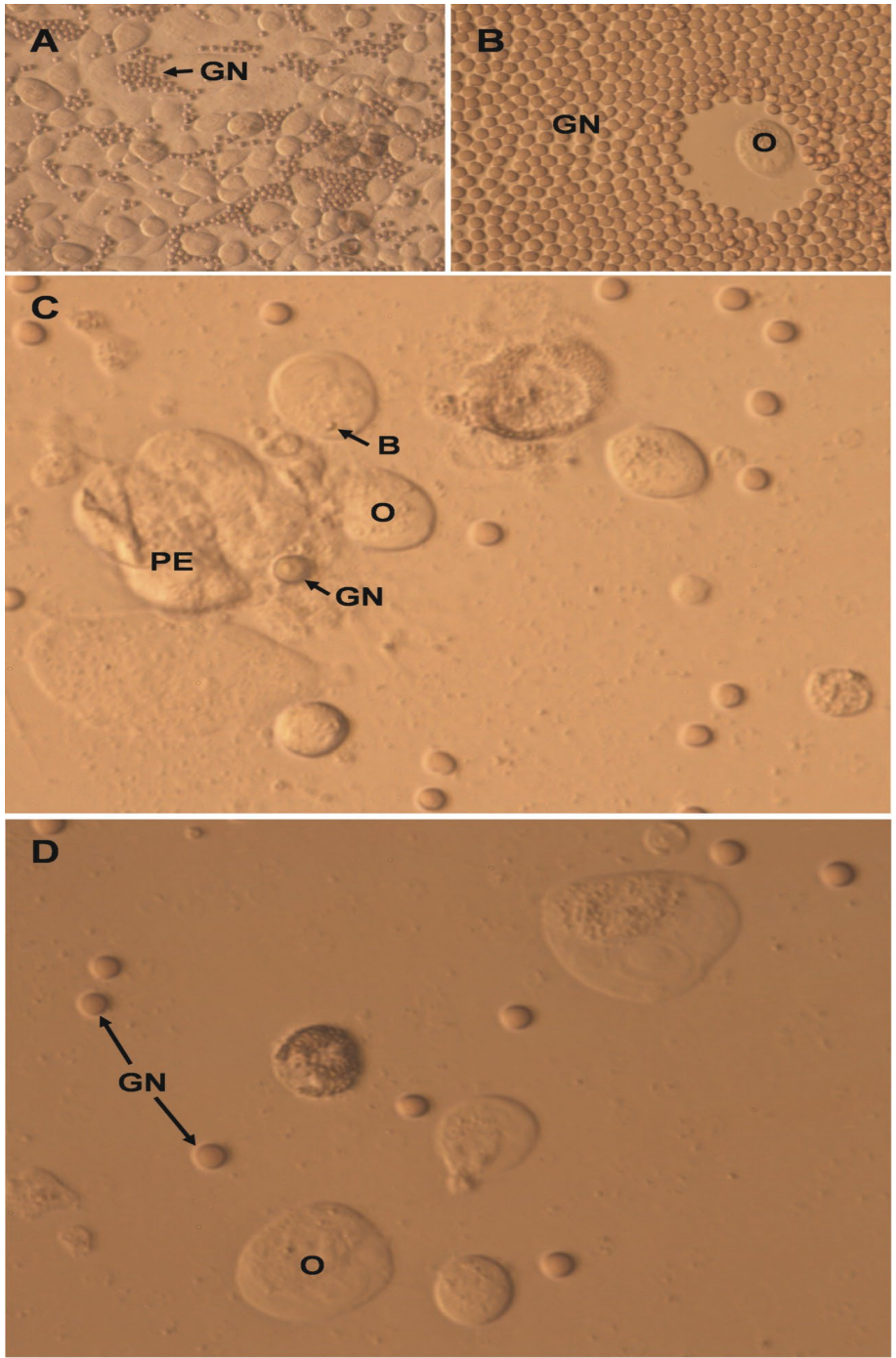

A
2 µm
B
E
ER
PSC
C
D
PSC
F
E

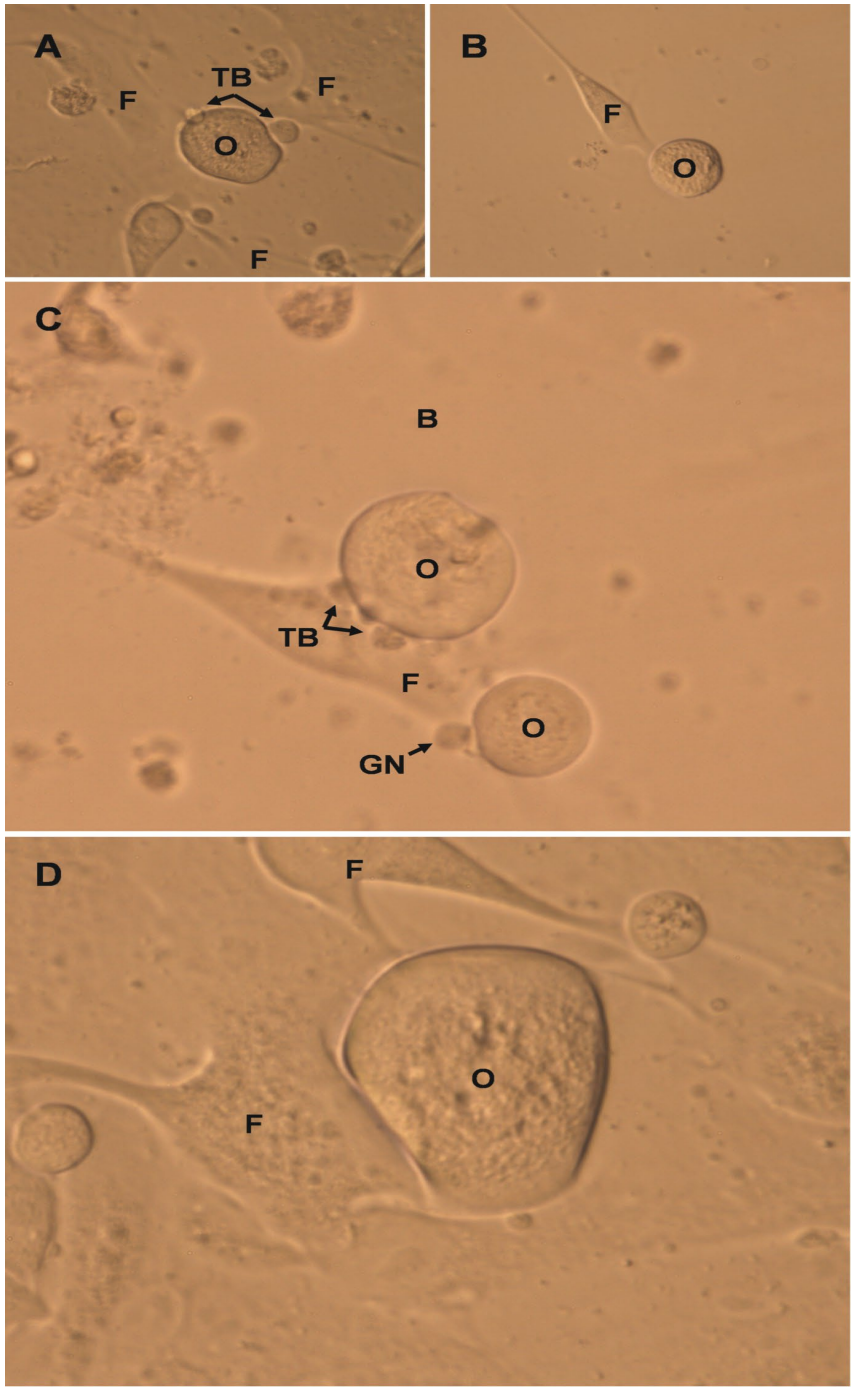

A
10 µm
B
20,49 µm

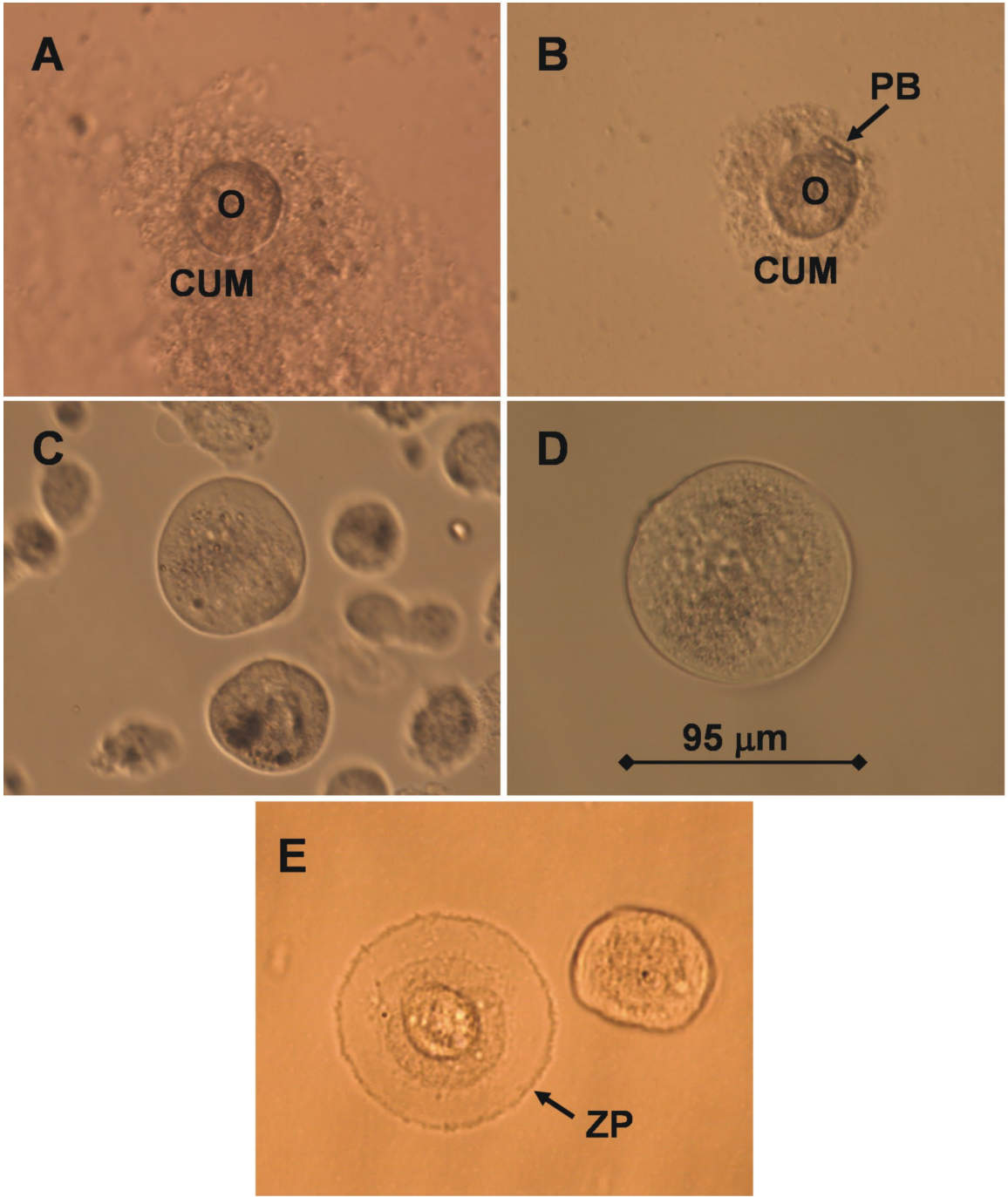

A
B

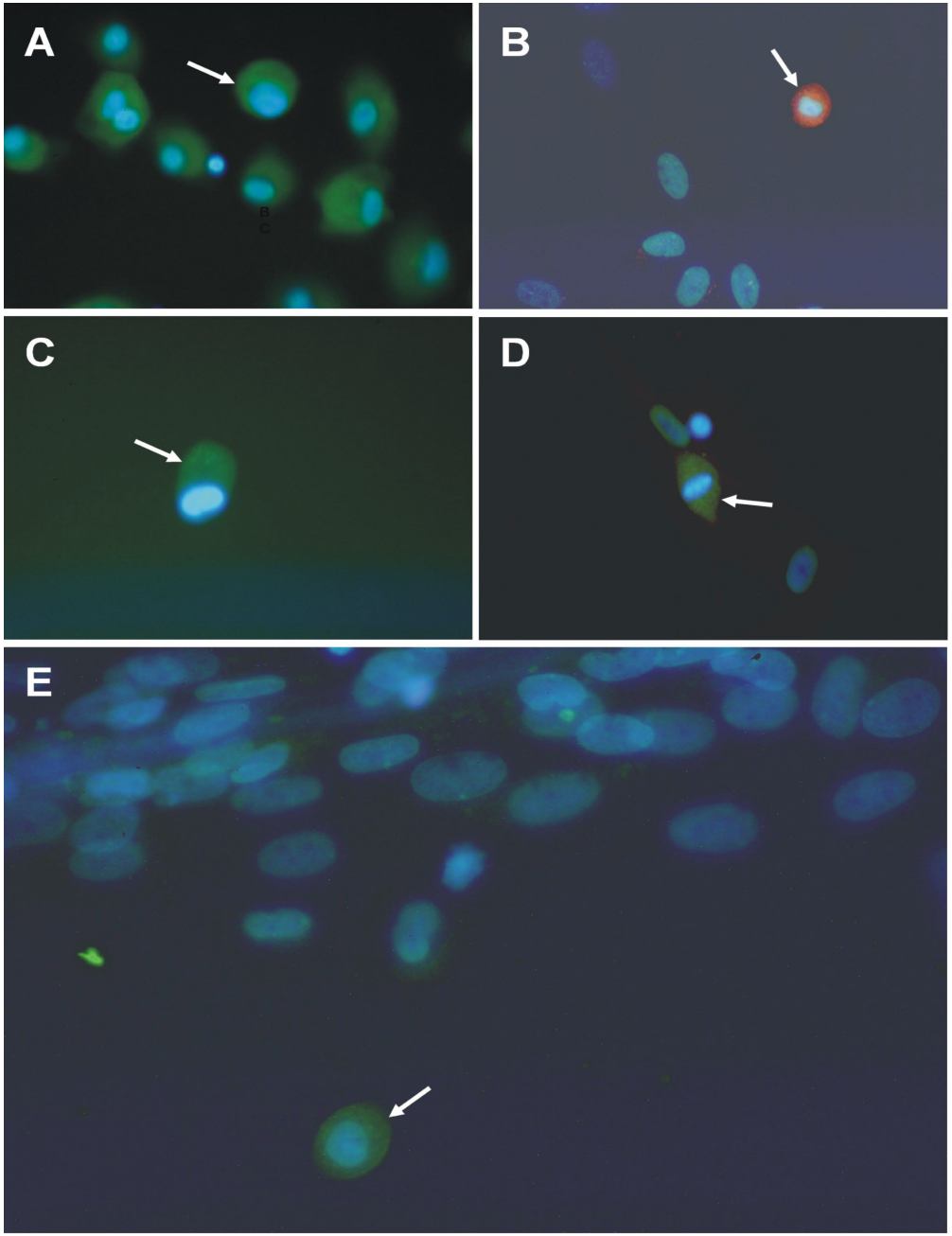

A
Nanog
285 bp
B
Sox-2
437 bp
C
Oct-4
315 bp
1
2
3
4
5
6

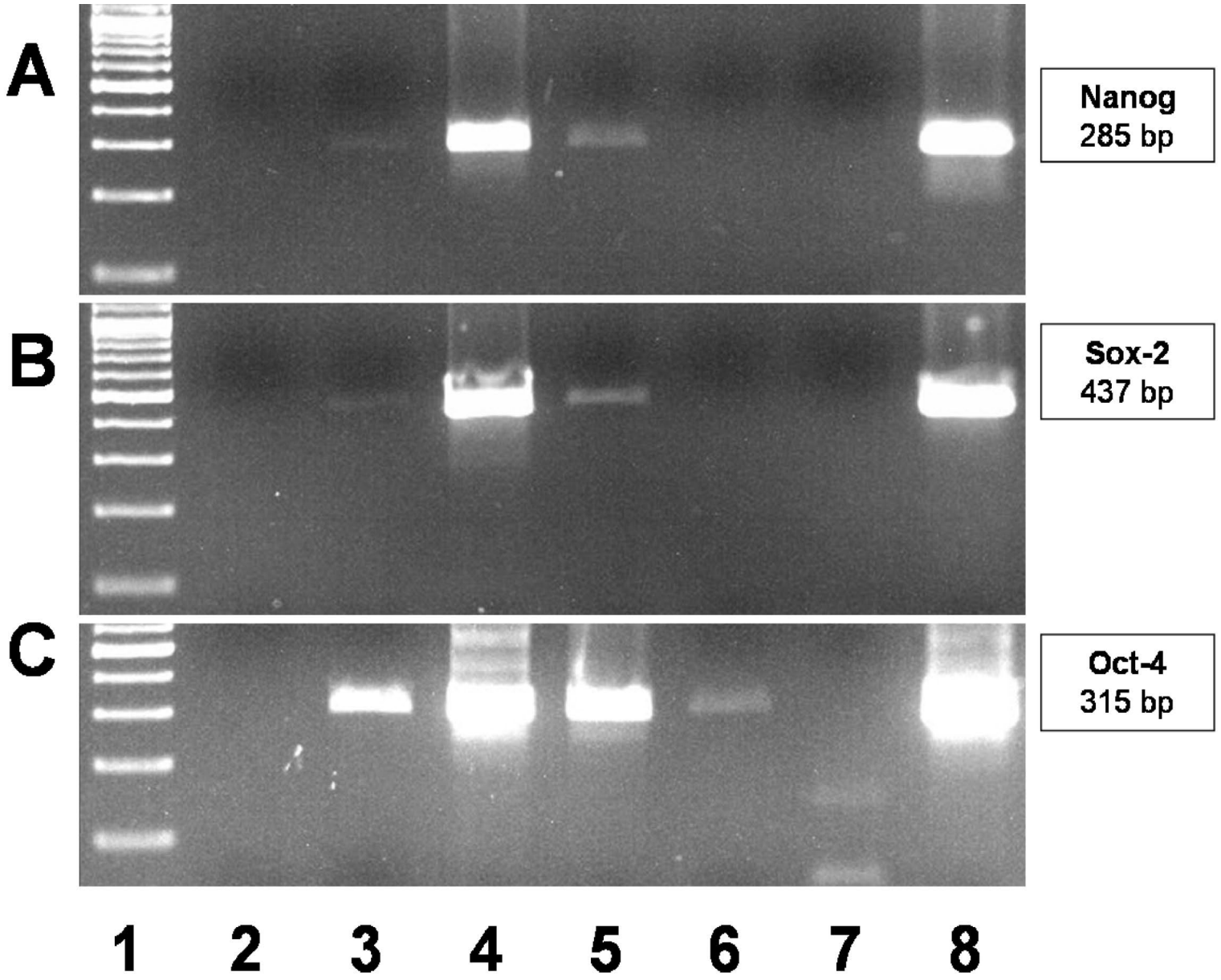

Day 0
Counts
M1
M2
SSEA-4
Day 0
SSEA-4
Day 20
Counts
M1
M2
SSEA-4
Day 20
SSC-H
SSEA-4


A
GN
B
GN
O
C
B
O
PE
GN
D
GN
O


A
TB
F
F
O
F
B
F
O
C
B
O
TB
F
O
GN
D
F
O
F


A
O
CUM
B
PB
O
CUM
C
D
95 µm
E
ZP


A
B
C
D
E


A
B
C
Nanog
285 bp
Sox-2
437 bp
Oct-4
315 bp
1
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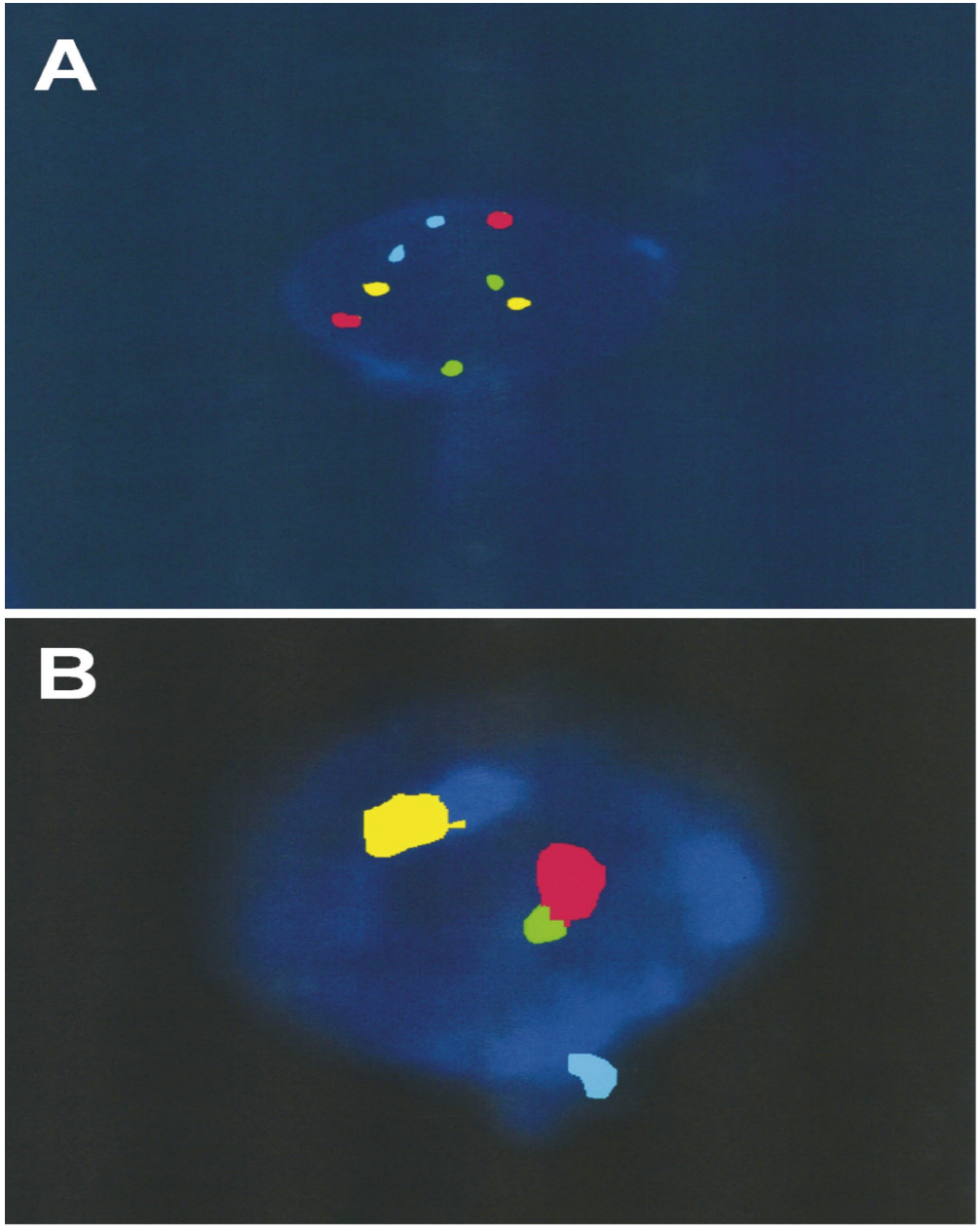
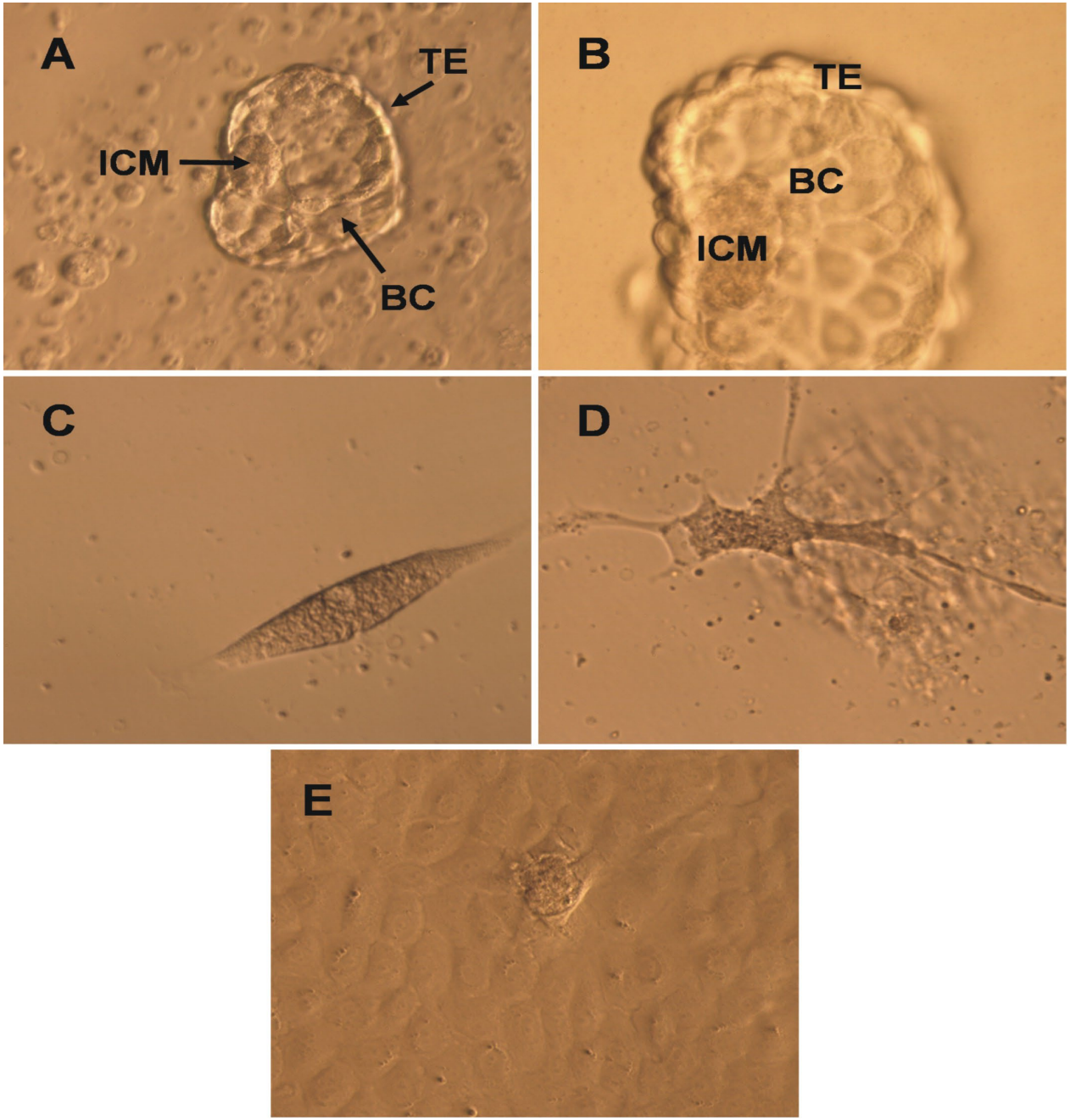
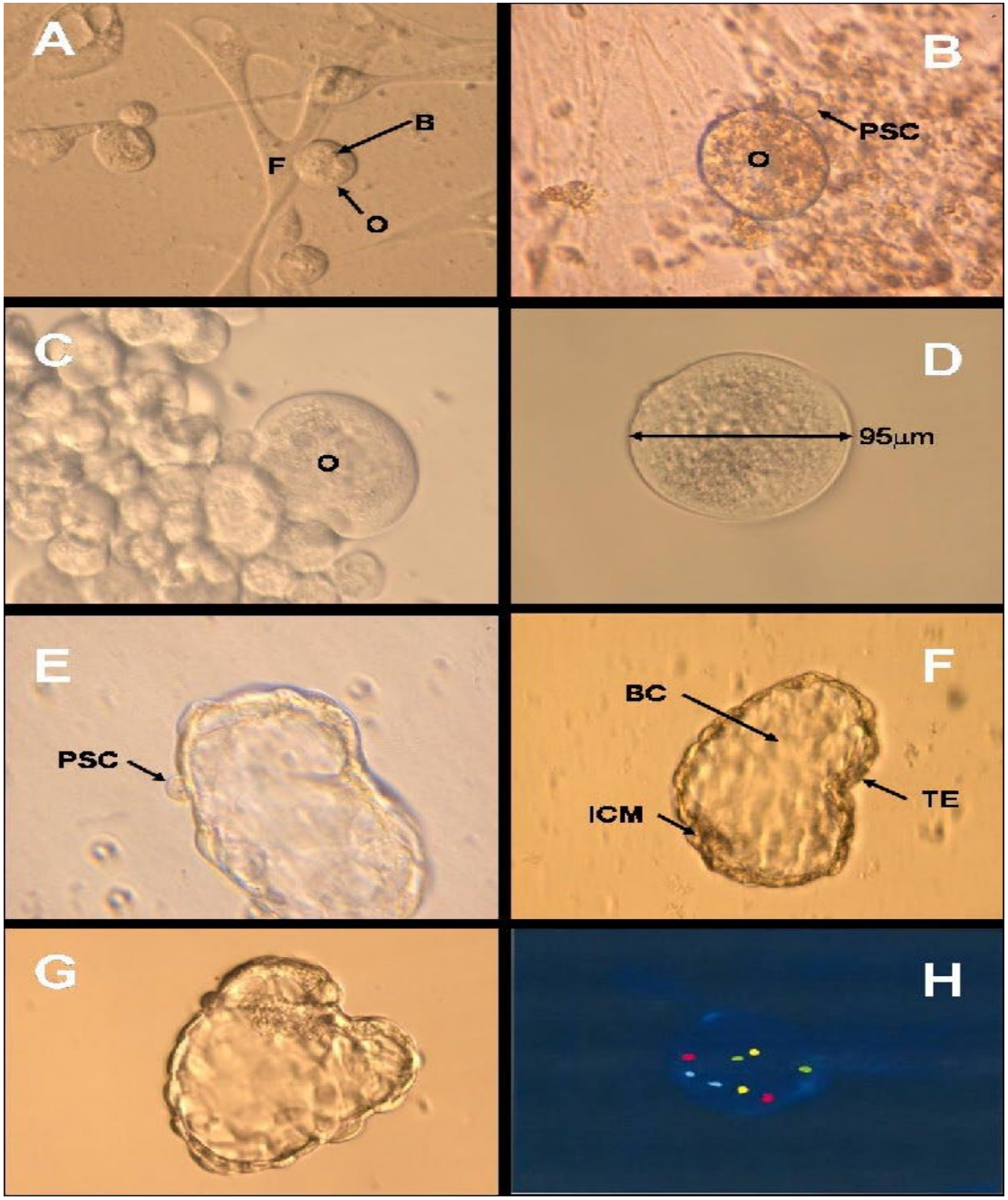
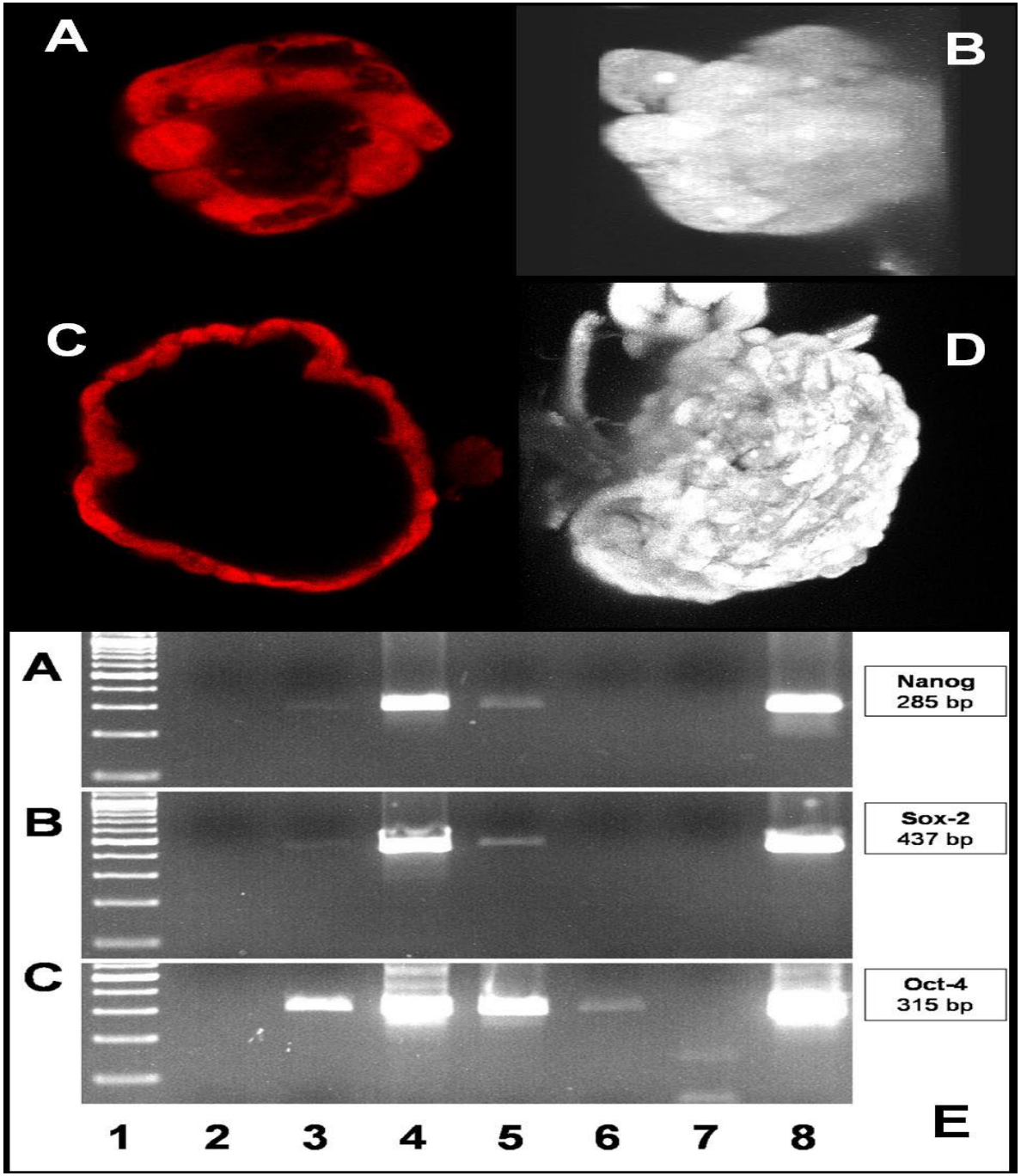
7
8


A
B


A
TE
ICM
BC
B
TE
BC
ICM
C
D
E


A
B
F
O
B
PSC
O
C
O
D
95µm
E
PSC
F
BC
TE
ICM
G
H


A
B
C
D
A
Nanog
285 bp
B
Sox-2
437 bp
C
Oct-4
315 bp
1
2
3
4
5
6
7
8
E

# Publikacije

Parthenogenetic Embryo-Like Structures in the Human Ovarian Surface Epithelium Cell Culture in Postmenopausal Women with No Naturally Present Follicles and Oocytes.

**Virant-Klun I**, Rozman P, Cvjeticanin B, Vrtacnik-Bokal E, Novakovic S, Ruelicke T

Stem Cells Dev. 2008 Jul 7. [Epub ahead of print]

Putative stem cells with an embryonic character isolated from the ovarian surface epithelium of women with no naturally present follicles and oocytes.

Virant-Klun I, Zech N, Rozman P, Vogler A, Cvjeticanin B, Klemenc P, Malicev E, Meden-Vrtovec H.Differentiation. 2008 Oct;76(8):843-56.

Study origin of germ cells and formation of new primary follicles in adult human and rat ovaries.

Bukovsky A, Gupta SK, **Virant-Klun I**, Upadhyaya NB, Copas P, Van Meter SE, Svetlikova M, Ayala ME, Dominguez R. Methods Mol Biol. 2008;450:233-65.

Ovarian germ cells.

Bukovsky A, Virant-Klun I, Svetlikova M, Willson I. Methods Enzymol. 2006;419:208-58. Review.

Potential new strategies for the treatment of ovarian infertility and degenerative diseases with autologous ovarian stem cells.

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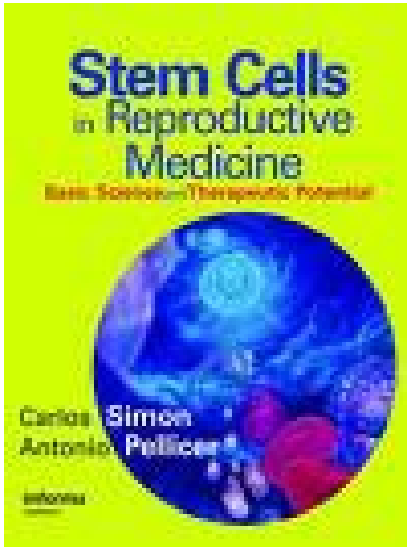
# Publikacije




QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Najnovejše spoznanje

# Okrogle spermatide