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Clinical Application of Human Oocyte Cryopreservation

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- *Principles of Oocyte Cryopreservation*
- *Current Status of Human Oocyte Cryopreservation*
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From Cryotherapy to Modern Cryobiology

- Egyptians, 2500 BC – to use low temperatures to stop bleeding, inflammation, & create local anesthesia

- Sir Robert Boyle at 15th century – "New Experiments and Observations Touching Cold"
  → "Boyle's law"

- Luyet & Gehenio in 1940 – "Life and Death at Low Temperature"

From Cryotherapy to Modern Cryobiology

➢ Polge et al., 1949 – "Revival of spermatozoa after vitrification and dehydration at low temperature"

≈

Cell injury associated
with subzero temperature exposure

≈

➢ Whittingham et al., 1972 – "Survival of mouse embryos frozen to -196℃ and -269℃"

History of Mammalian Oocyte Cryopreservation

- Chang et al., 1952 – "Fertilizability of rabbit ova and the effects of temperature in vitro on their subsequent fertilization and activation in vivo"

- Leibo et al., 1978 – Cell survival $\propto$ cooling rate & intracellular ice formation

  -> slow cooling for cryopreserving oocytes

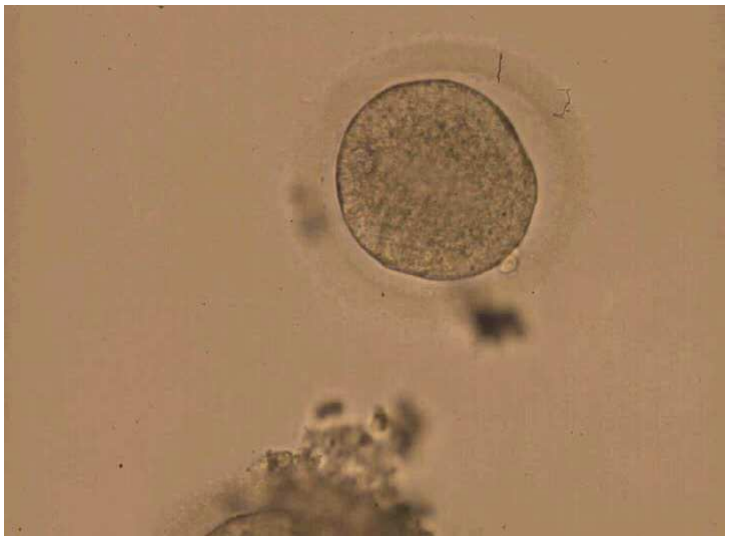
History of Human Oocyte Cryopreservation

- ***Porcu et al. 2008*** *1st healthy twins after oocyte cryopreservation and bilateral ovariectomy for ovarian cancer*
- ***Yang et al. 2007*** *First healthy male c frozen oocytes of a HD patient*
- ***Cha et al./Kuleshova et al. 1999*** *The first pregnancy and birth from vitrified oocytes*
- ***Chen et al. 1986*** *First healthy newborn from slowly frozen oocyte*

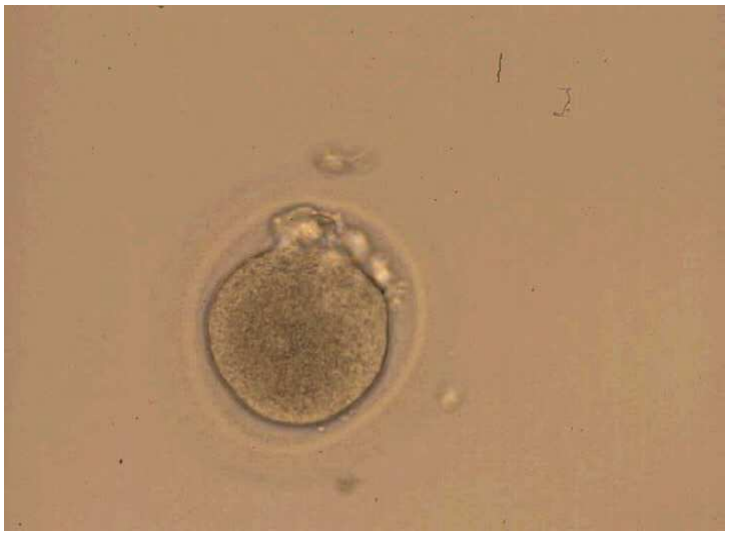
Principles of Oocyte Cryopreservation

Vitrification

Equilibration Procedure



Pre-equilibration
EG 1.5 M
For 2.5 min



Equilibration
EG 5.5 M + 1.0 M sucrose
For 20 seconds

Vitrification

High cooling rate

- Minimizing the volume of CPA solution

  - Using special carriers; EM grid (Martino et al., 1996), Open Pulled Straws (Vajta et al.,1998), Cryoloop (Lane et al., 1999), Hemi-straw (Vanderzwalmen et al., 2003), Cryotop (Kuwayama et al., 2005)

- Slush Nitrogen (Yoon et al., 2007)

Vitrification

High concentration of cryoprotectant agent (CPA) solution

- Mechanism of the protective action of CPA is same

  but : toxicities, permeability are different

- Osmotic change before and after cryopreservation

  → cause the death of cells

- Ethylene glycol (EG) only : low toxicity & rapid permeation

- EG + other CPA (DMSO, 1,2-PrOH …)

  → reduce concentration of single CPA

  → decrease the individual specific toxicity

  (Cha et al.,2011)

Current Status of Human Oocyte Cryopreservation

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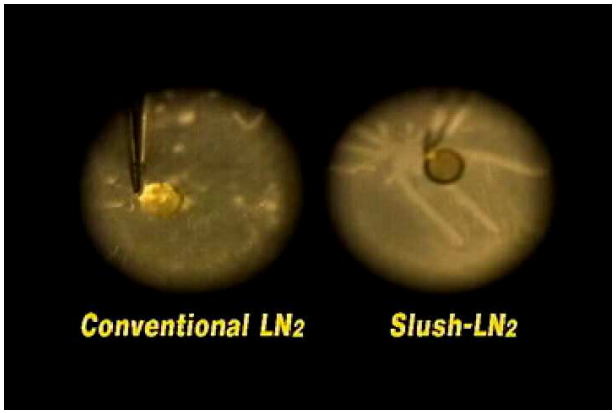
Slush Nitrogen (SN2)

Application of negative pressure w/ vacuum → LN_2 → SN_2

(mixture of liquid & solid nitrogen)

Liquid Nitrogen (LN2) vs. Slush Nitrogen (SN2)



	LN_2	SN_2
Temperature	-196°C	-210°C
Vaporization	Yes	No
Cooling Rate	-20,000°C / min	-135,000°C / min
CPA Concentration	High	Low
Cryo-damage	low	very low

Clinical Outcomes of IVF-ET Programs Using Vitrified Human Oocytes from Stimulated Cycles by SN2

	SN_2
No. of Cycles	30
No. of Vitrified / Thawed Oocytes	364
No. of Survived Oocytes (%) [a]	302 (82.9 ± 2.9)[b]
No. of Injected oocytes	218
No. of Fertilized Oocytes (%)	168 (77.1 ± 3.5) [b]
No. of Cleaving 2PN Embryos (%)	158 (94.0 ± 2.1) [b]
No. of Cycles Undergoing ET	30 (100)
Pregnancies (%/ET)	13/30 (43.3)
Miscarriage (%)	2 (15.4)
Delivery/ongoing	4[c]/ 7
No. of transferred embryos	120 (4.0 ± 0.2) [b]
Implantation Rate (%)	17 (14.2)

[a] No. of intact oocytes after warming (%/vitrified oocytes)
[b] Mean ± SEM
[c] Three singleton (male/male/female), one twin (male/male)

(Yoon et al., Fertil Steril 2007)

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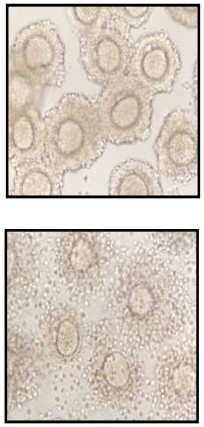
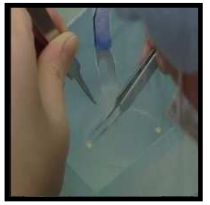
Procedure of oocyte vitrification



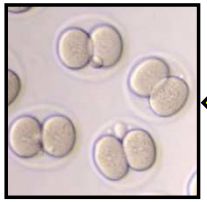
LN_2	SN_2
5.5M EG + 1M sucrose	5.5M EG + 1M sucrose
7.5%EG+7.5%DMSO+0.5M sucrose	7.5%EG+7.5%DMSO+0.5M sucrose
10%EG+10%DMSO+0.5M sucrose	10%EG+10%DMSO+0.5M sucrose
15%EG+15%DMSO+0.5M sucrose	15%EG+15%DMSO+0.5M sucrose

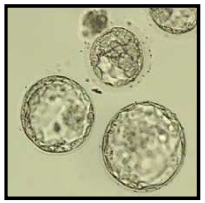


1.0M sucrose
0.5M sucrose
0.2M sucrose
0.125M sucrose

In vitro maturation

In vitro fertilization





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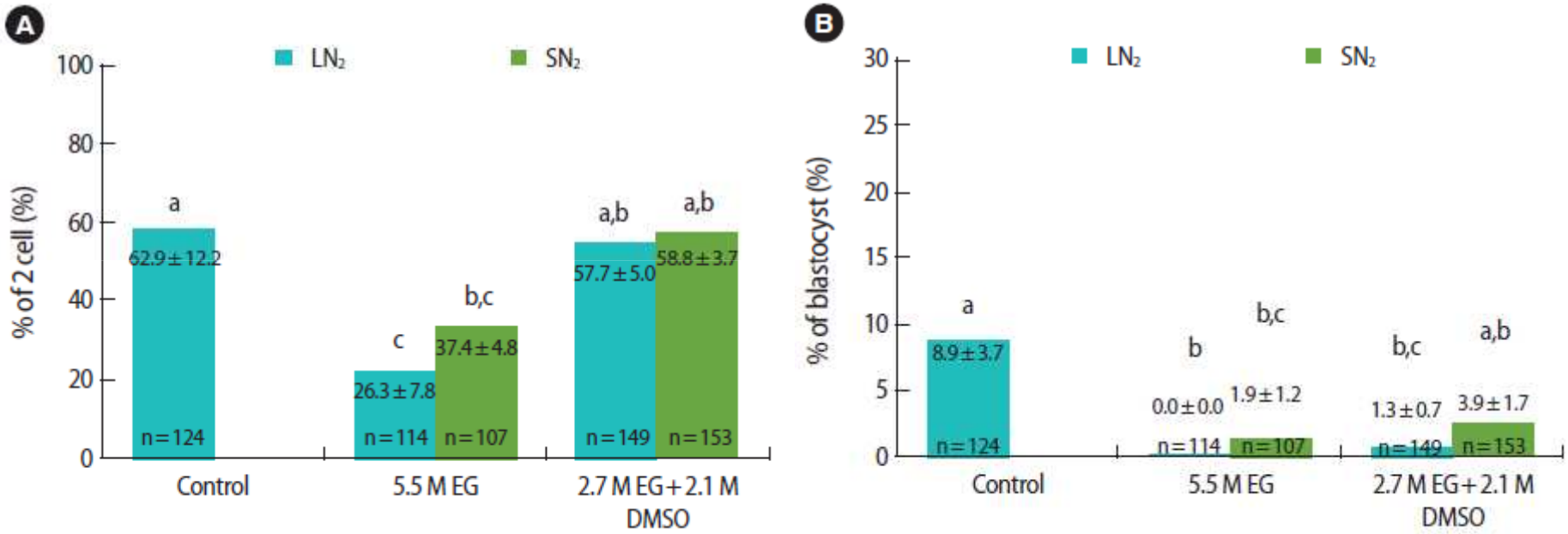
The effect of different cryoprotectants & cooling speed of immature oocytes



Cleavage Stage

Blastocyst Stage

(Cha et al., 2011)

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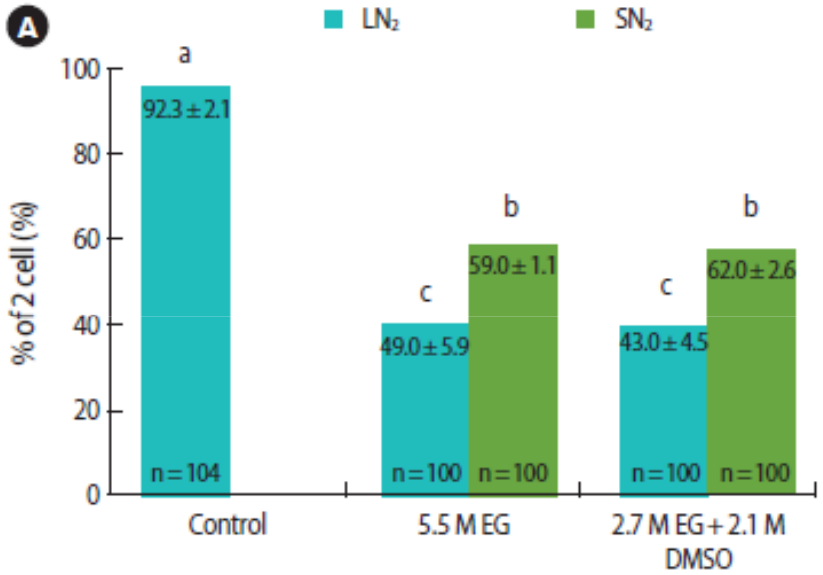
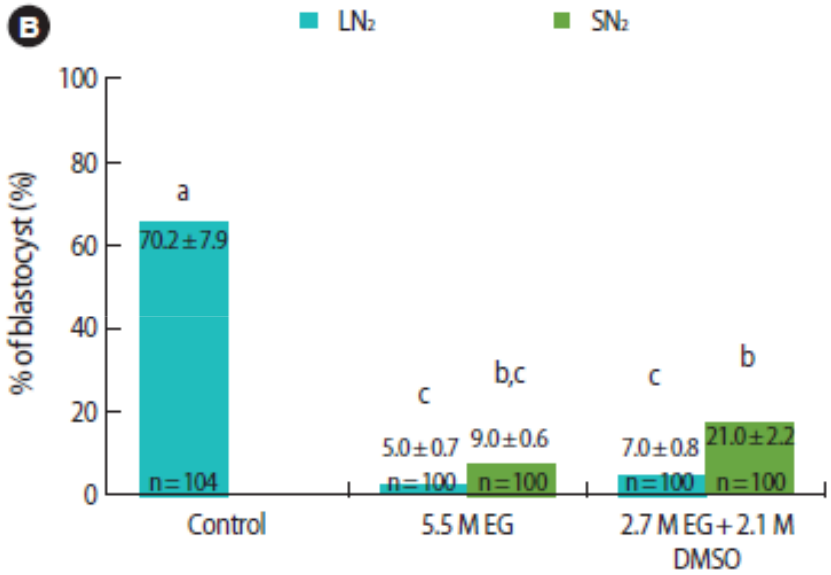
The effect of different cryoprotectants & cooling speed of mature oocytes



Cleavage Stage



Blastocyst Stage

(Cha et al., CERM, 2011)

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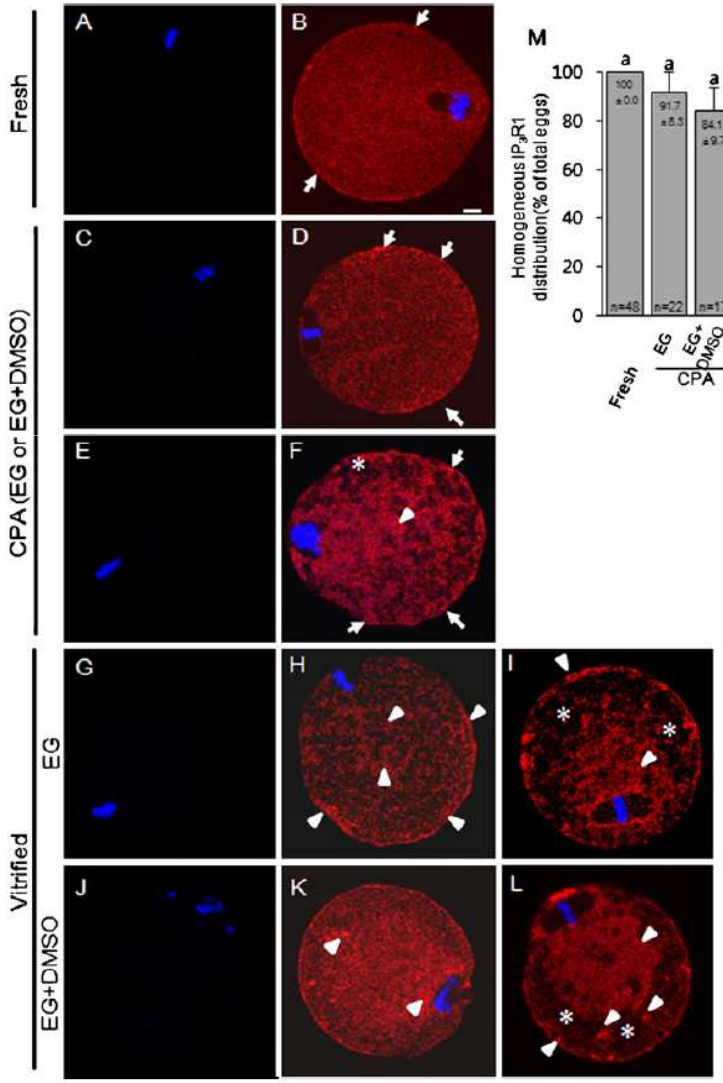
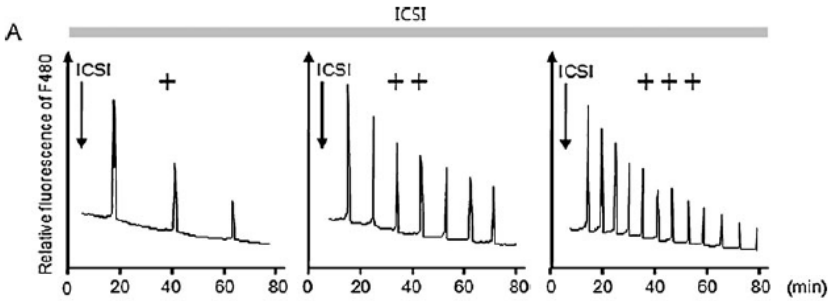
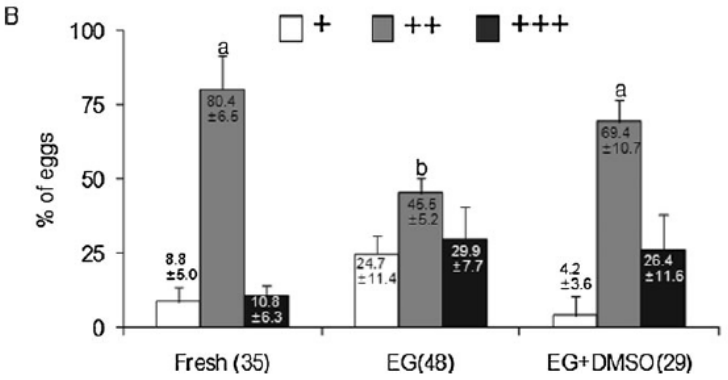
Alterations in calcium oscillatory activity in vitrified eggs







(Kim et al., Eur J Physiol, 2011)

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Alterations in calcium oscillatory activity in vitrified eggs

Table 2 Embryonic development rates of fresh and vitrified/warmed eggs after ICSI

Group	Number of injected eggs	Number intact eggs after ICSI	Number two-cell embryos (%/intact)	Number morulae (%/two-cell)	Number blastocysts (%/two-cell)
Fresh	107	52 (47.9±4.2)[a]	49 (92.5±5.6)[a]	45 (92.3±3.9)[a]	39 (80.3±5.2)[a]
EG	215[c]	106 (48.2±2.5)[a]	100 (93.3±4.2)[a]	73 (72.8±1.8)[b]	51 (48.8±4.8)[b]
Fresh	225	111 (49.8±1.8)[a]	106 (94.9±2.2)[a]	96 (88.7±3.6)[a]	78 (71.6±5.2)[a]
EG+DMSO	228[c]	136 (59.4±3.3)[b]	130 (96.1±1.9)[a]	115 (88.4±3.9)[a]	93 (73.2±5.3)[a]

Data are shown as mean (%) ± SEM for five replications (EG group) and seven replications (EG+DMSO group)

[a, b] Within the same column with different superscripts represent significant differences ($P<0.05$)

[c] The number of survived eggs after vitrification and warming.

(Kim et al., Eur J Physiol, 2011)

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First baby born from vitrified oocytes

(Yoon et al, Fertil Steril, 2003)

Oocyte Banking: Cancer patients

Oocyte banking for cancer patients

(Fertility Center of CHA Gangnam Medical Center, July 1999-August 2011)

Diagnosis	No. of Patients	Mean Age (Year)	Retrieved	Mature
CML	13	26.4 ± 5.5	14.2 ± 9.9	10.8 ± 7.6**
MDS	5*	29.0 ± 2.3	14.4 ± 6.7	10.0 ± 6.0
AML	2	22.5 ± 0.5	25.5 ± 9.5	13.0
ALL	1	27.0	19.0	12.0
HD	1	18.0	9.0	5.0
Lymphoma	3	20.7 ± 1.2	19.7 ± 0.9	14.0 ± 0.9
Rectal	3	28.0 ± 1.2	23.3 ± 13.2	14.0 ± 12.3
Thyroid Ca	1	24.0	14.0	4.0
Breast Ca	4	30.8 ± 3.6	9.5 ± 3.9	6.0 ± 2.3
(Lupus)	1	29.0	1.5	1.5
Endometrial stromal sarcoma	1	38.0	44.0	26.0
Mucinous liposarcoma	1	31.0	11.0	6.0

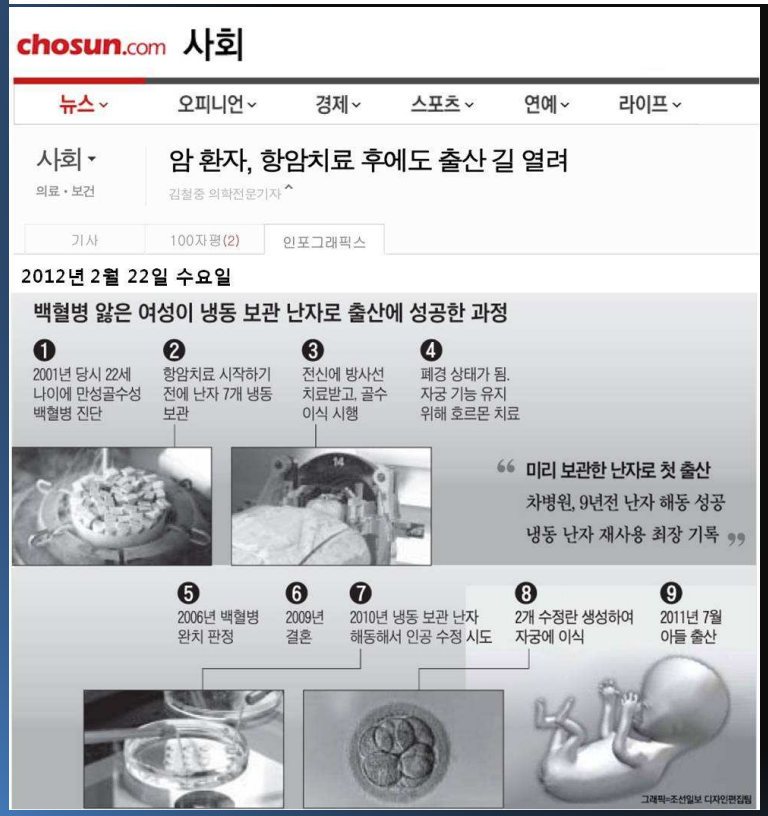
* Embryo Freezing (n=2), ** Immature Oocyte (n=1)

Oocyte Banking

FERTILITY PRESERVATION

Live birth with vitrified-warmed oocytes of a chronic myeloid leukemia patient nine years after allogenic bone marrow transplantation

Mi Kyoung Kim · Dong Ryul Lee · Ji Eun Han · You Shin Kim · Woo Sik Lee · Hyung Jae Won · Ji Won Kim · Tae Ki Yoon



- *Oocytes of cancer patients stored over the long-term can successfully develop to the in vitro cleavage stage and result in a live birth.*

(Kim et al., J Assit Reprod Genet, 2011)

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Applications of Oocyte cryopreservation

A successful method to maintain fertility for cancer pts & pts who are faced with losing their ovarian function

Emergency oocyte freezing in the case of incidental azospermia

Fertility preservation for old single women as getting enormously increased late marriage

Alternative method for storing the excess oocytes & avoiding the development of more embryos

Oocyte banking for oocyte donor program

Conclusion

In spite of excellent clinical results of IVF with vitrified/warmed oocytes, there are low enrollments of fertility preservation program of cancer patients.

Fertility specialist should be cooperated more actively with oncologists.

All cancer patients who are faced with losing their fertility should be informed about fertility preservation.

For old single women & POF patients, social reconsideration about oocyte freezing & oocyte banking is requested.

Further studies & improvements are necessary to imply this technique in human assisted reproductive technology.