

A Combination of Simvastatin and Methylprednisolone Improve the Quality of Vitrified-Warmed Ovarian Tissue after Transplantation

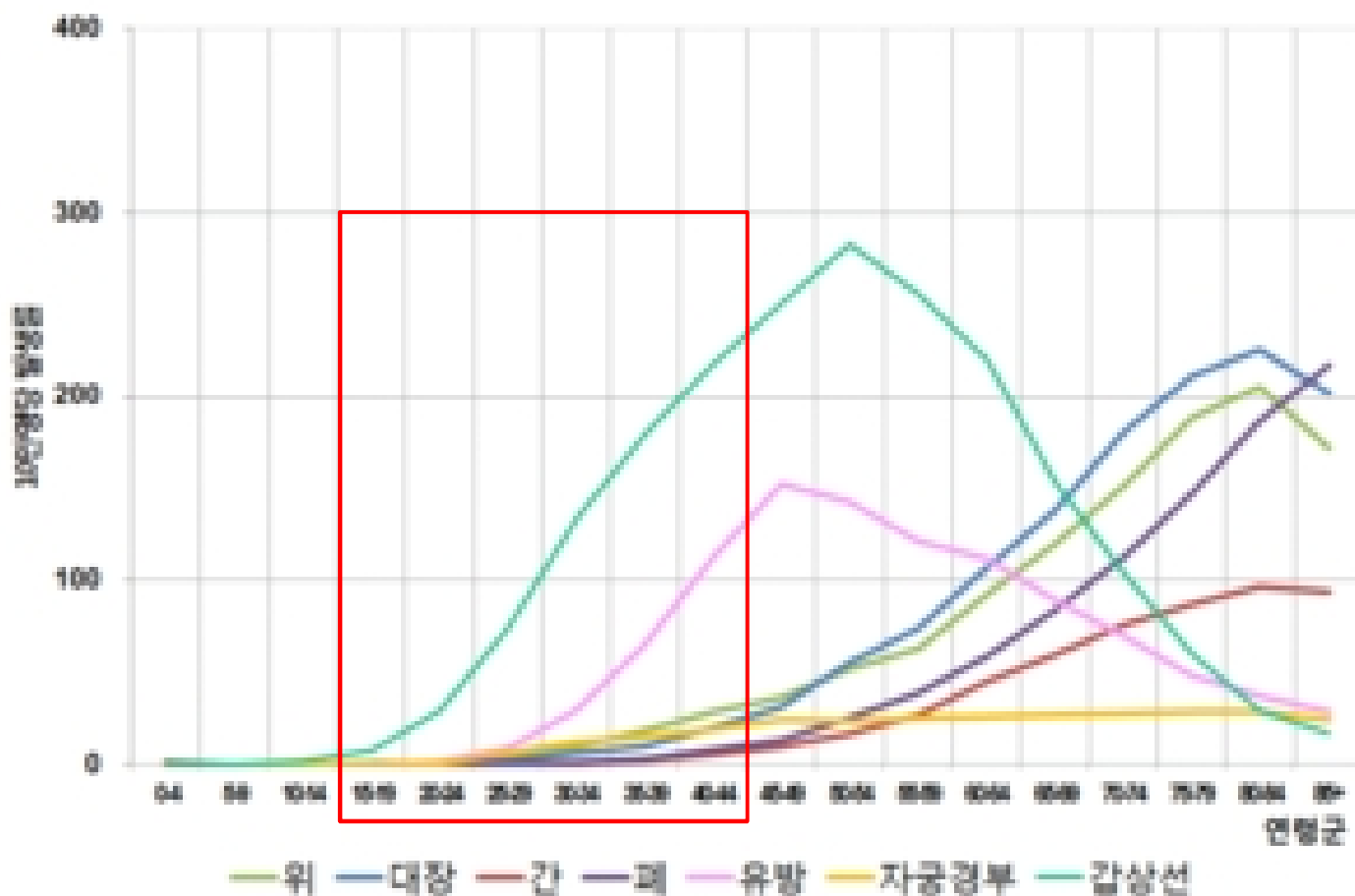
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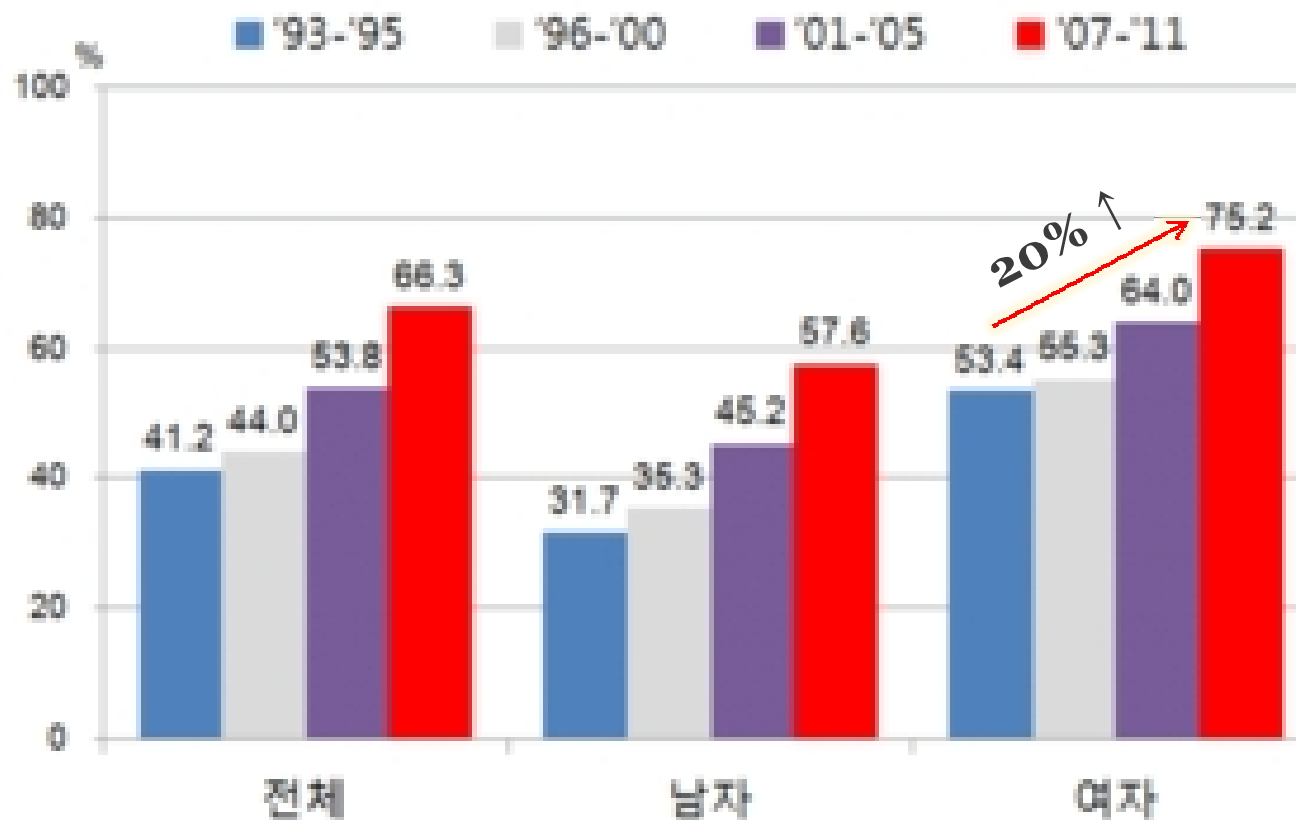
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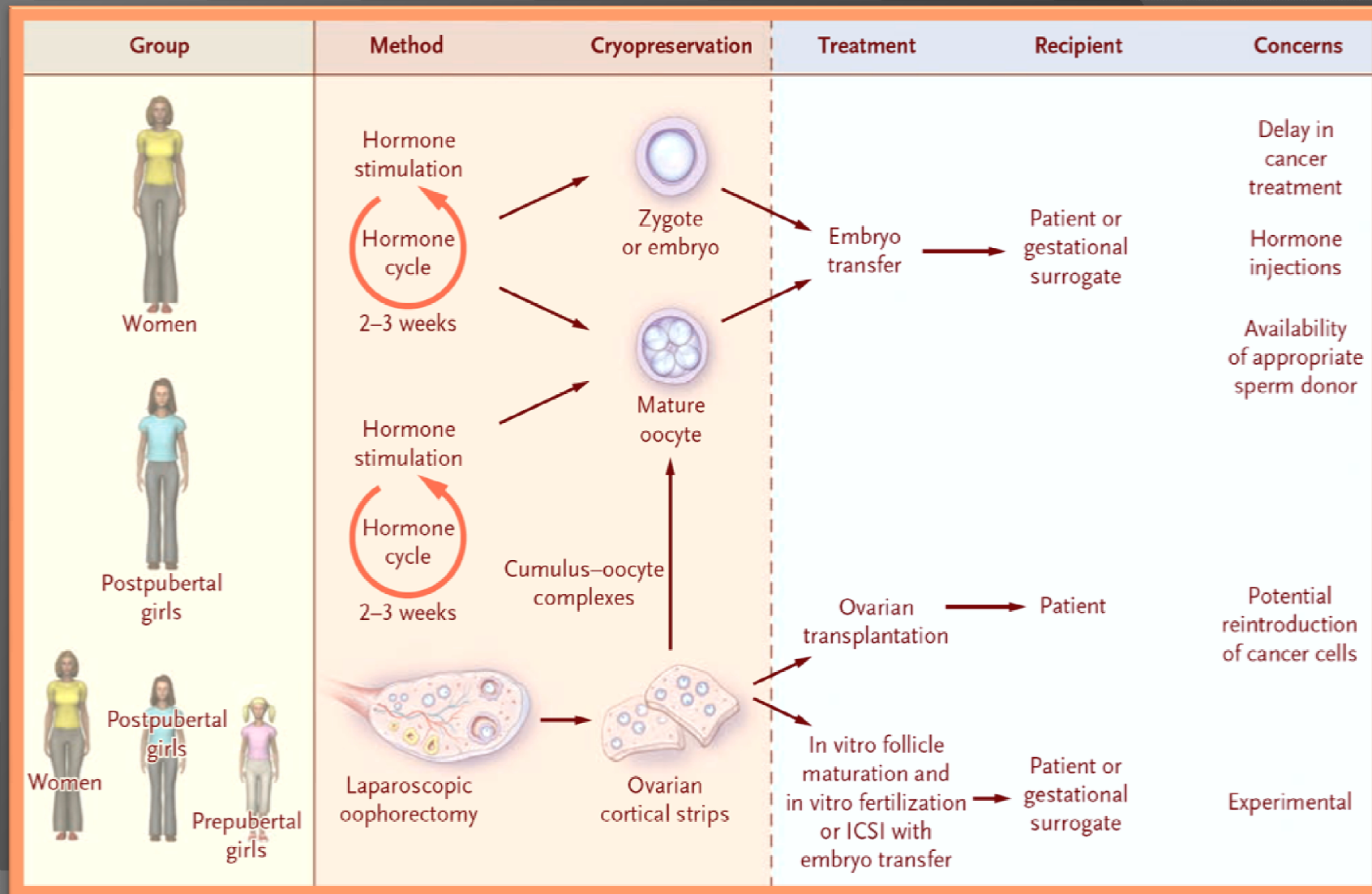
주요 암종 연령군별 발생률: 여자, 2011



성별 5년 생존율 추이: 모든 암



Options for Fertility Preservation in Female Cancer Patients



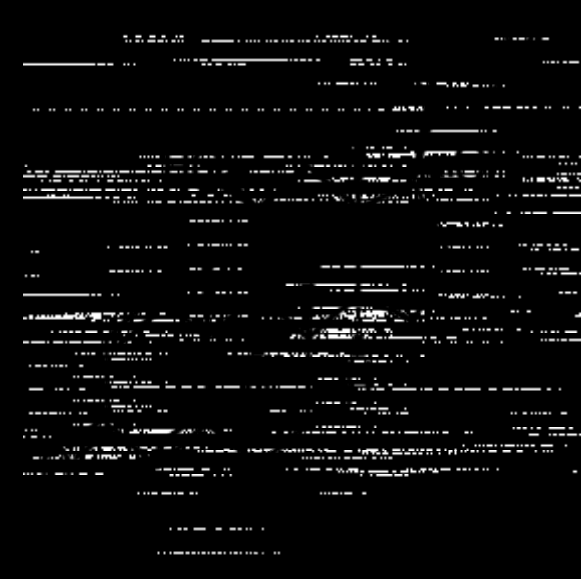
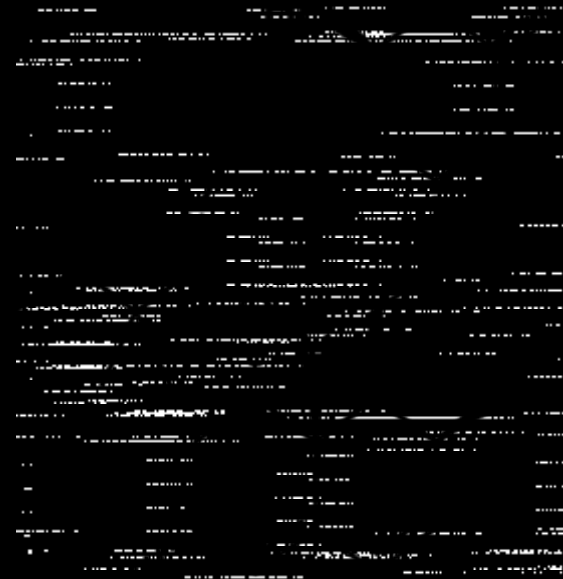
Problems during Ovarian Tissue Cryopreservation & Transplantation

- There are many **hurdles** during ovarian tissue (OT) cryopreservation and transplantation
 - Cryodamage (Cryopreservation)
 - **Ischemic injury (Transplantation)**
 - Re-implantation of malignancy cells (After transplantation)
- In general, after transplantation, ovarian revascularization occurs and numerous ovarian follicles are lost during the ischemic period (Israely et al., 2003, Jeremias et al., 2002)
- At least 25% of the primordial follicles are lost as a result of transplantation (TPL) of cryopreserved xenografts of human OT into mice (Newton et al., 1996).
- Therefore, **reducing the ischemic damage** during OT TPL is **important** for **successful tissue TPL**.

Simvastatin

Methylprednisolone

Chemical Structure



Main Function

•HMG-CoA reductase inhibitor

•Corticosteroid

Main Targets

•Hyperlipidemia
•Coronary artery disease

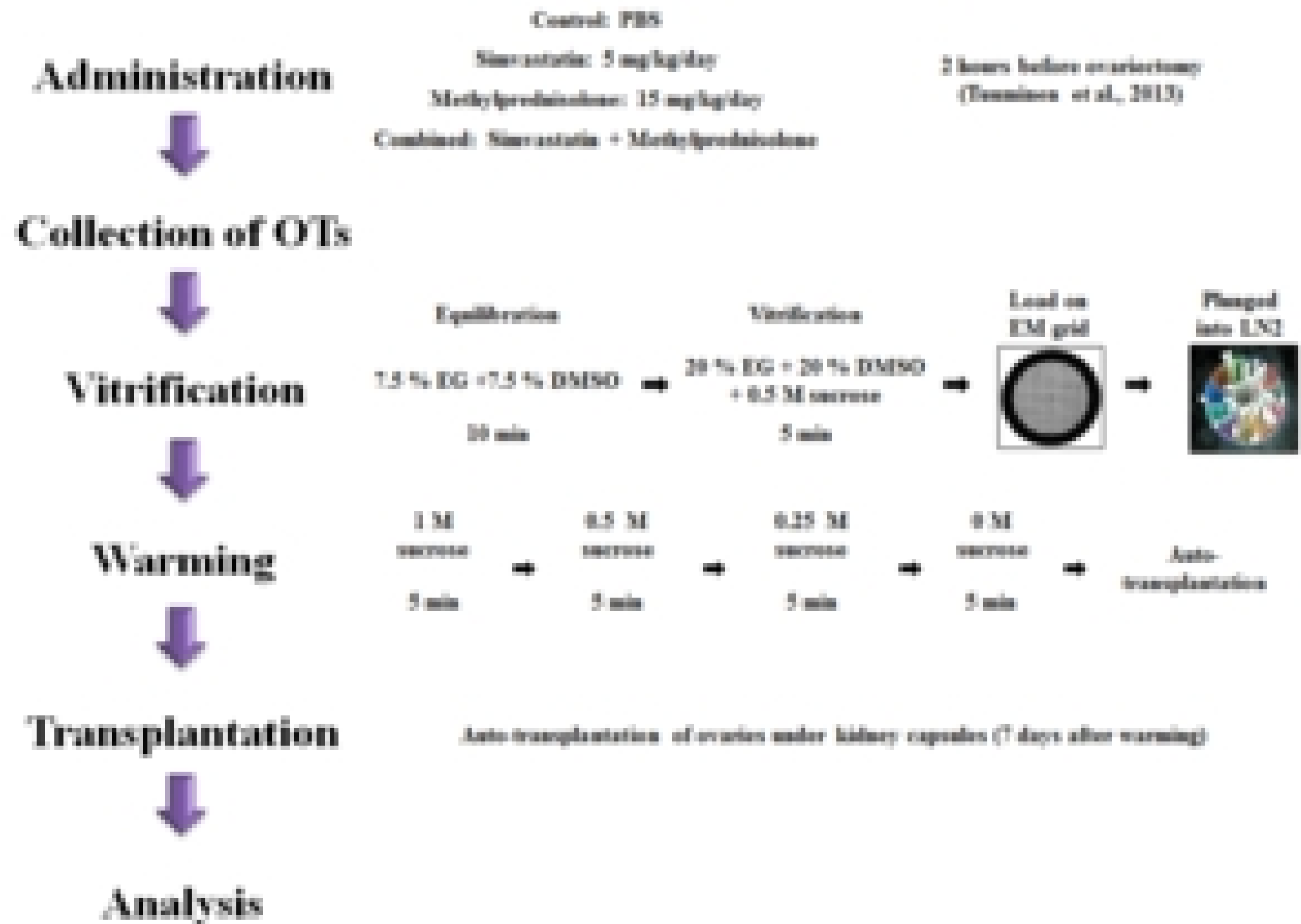
•Arthritis
•Auto-immunedisease

Other Effects

•Angiogenesis
•Anti-inflammation
•Inhibition of apoptosis

•Decrease nitric oxide (NO)

Materials & Methods



Materials & Methods

- **Animal**

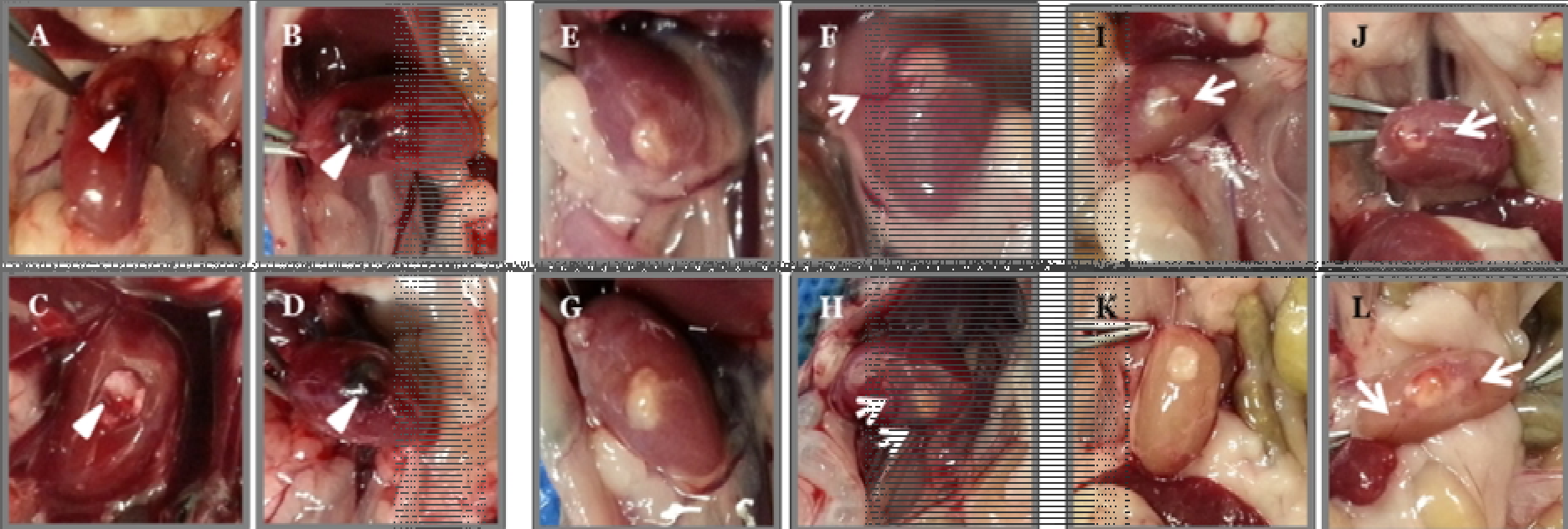
- ✓ 6-week-old aged BD F-1 mice
- ✓ Sacrifice on **day 2, 7 and 21** after transplantation

- **Assessment Methods**

- ✓ Gross Observation
- ✓ Histological Analysis – **H&E Stain**
- ✓ Follicle Classification – **Grade 1 Follicle**
- ✓ Apoptosis – **TUNEL Assay**
- ✓ Vessel Density – **IHC for CD31**
- ✓ Immune Cell Infiltration – **Flow Cytometry for CD45**
- ✓ Ovarian Reserve – **ELISA for Serum AMH**
- ✓ Embryonic Development – **OPU, IVF and DST**

Results

Gross Observation



Histological Assessment

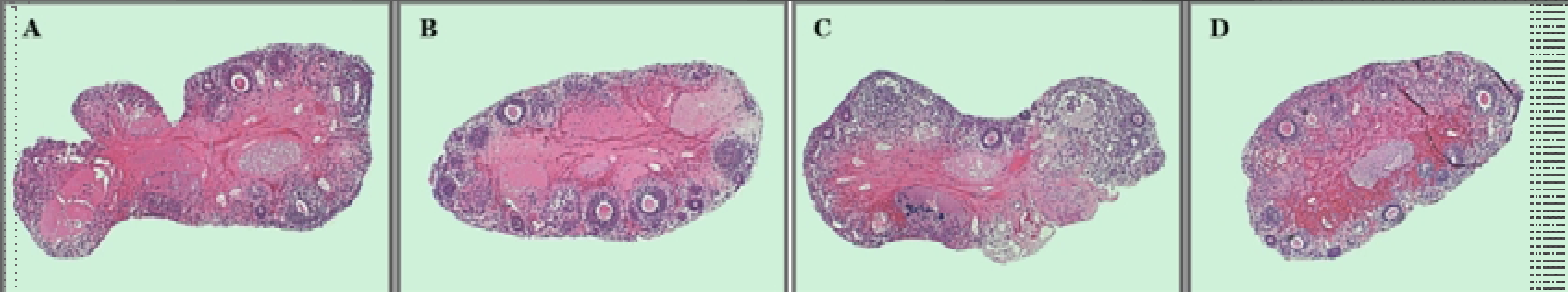
CON

Simv.

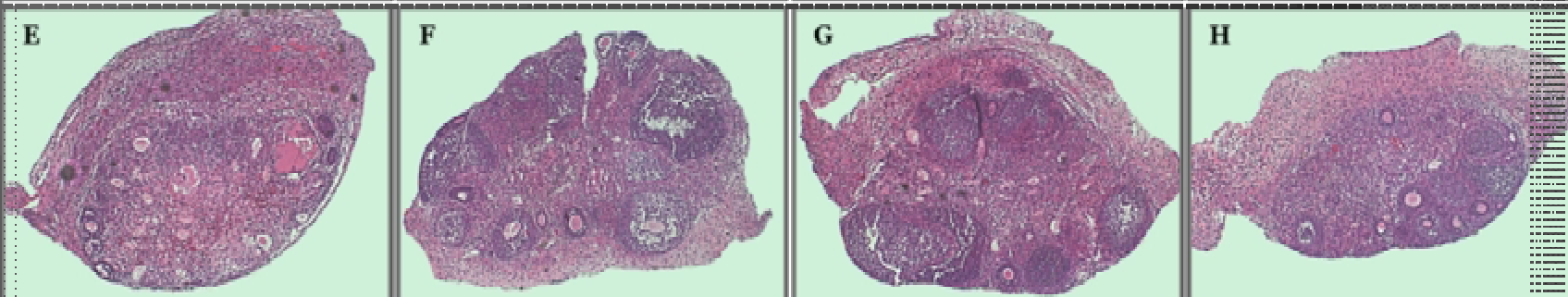
M.P.

S+M

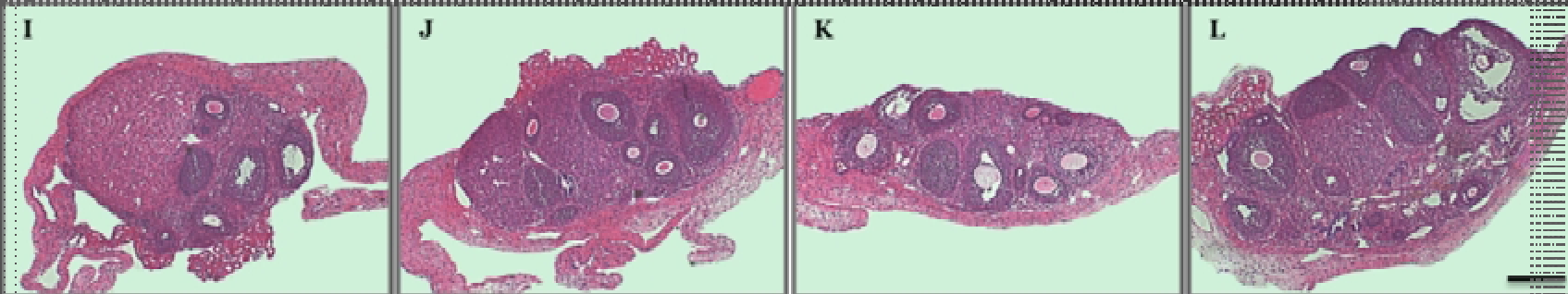
Day 2



Day 7



Day 21

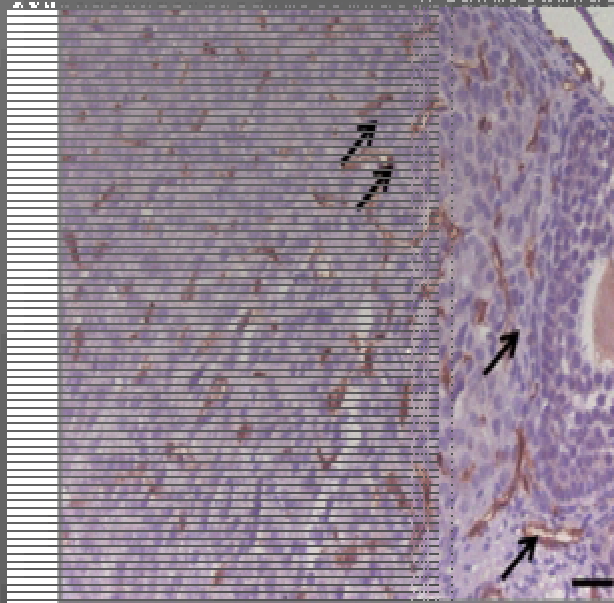


Grade 1 Follicle and Apoptotic Follicle Ratio

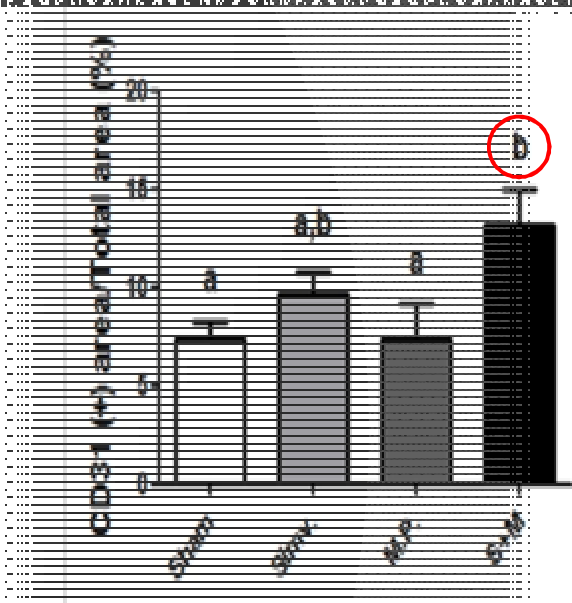
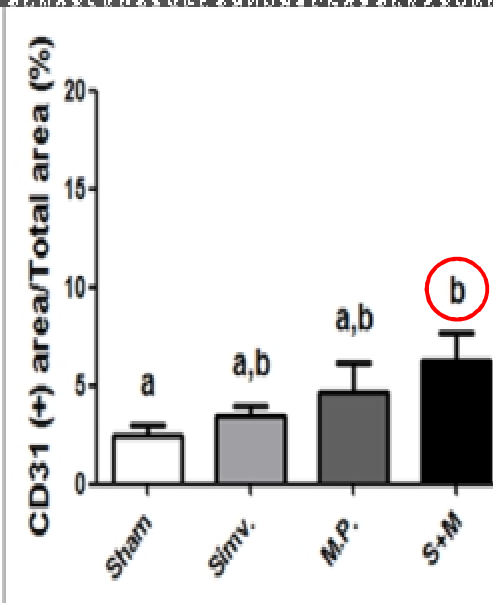
Table with 10 columns: Patient ID, Age, Grade 1 Follicle Count, Apoptotic Follicle Count, Ratio, and various clinical parameters. The table contains 10 rows of data with some cells highlighted in yellow.

Patient ID	Age	Grade 1 Follicle Count	Apoptotic Follicle Count	Ratio	Parameter 1	Parameter 2	Parameter 3	Parameter 4	Parameter 5
001	25	15	5	0.33	120	100	80	60	40
002	30	20	8	0.40	130	110	90	70	50
003	35	18	6	0.33	110	90	70	50	30
004	40	22	9	0.41	140	120	100	80	60
005	45	16	4	0.25	100	80	60	40	20
006	50	25	10	0.40	150	130	110	90	70
007	55	14	3	0.21	90	70	50	30	10
008	60	28	12	0.43	160	140	120	100	80
009	65	12	2	0.17	80	60	40	20	10
010	70	30	15	0.50	170	150	130	110	90

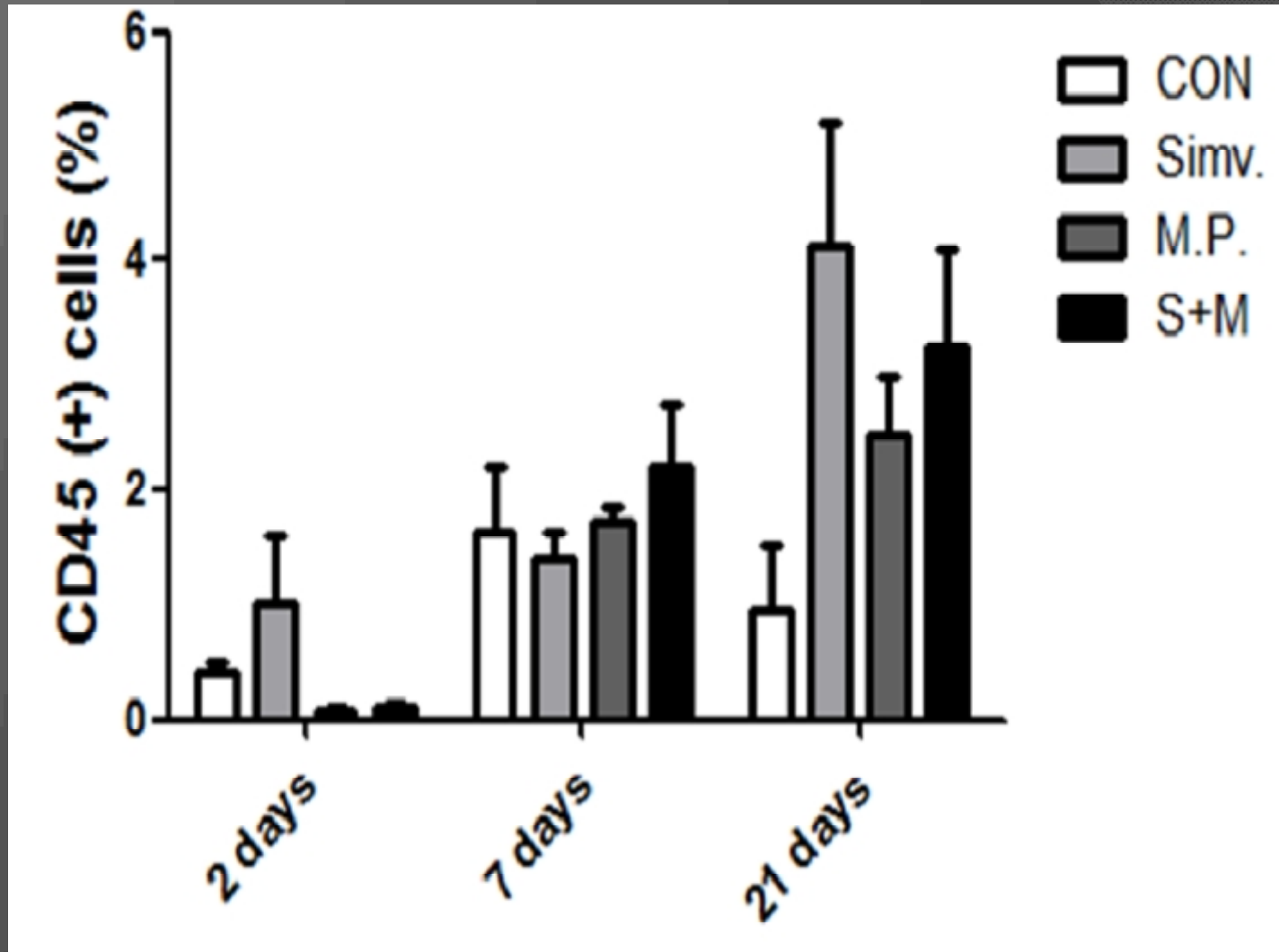
The Proportion of CD31 Area



→ Blood Vessels

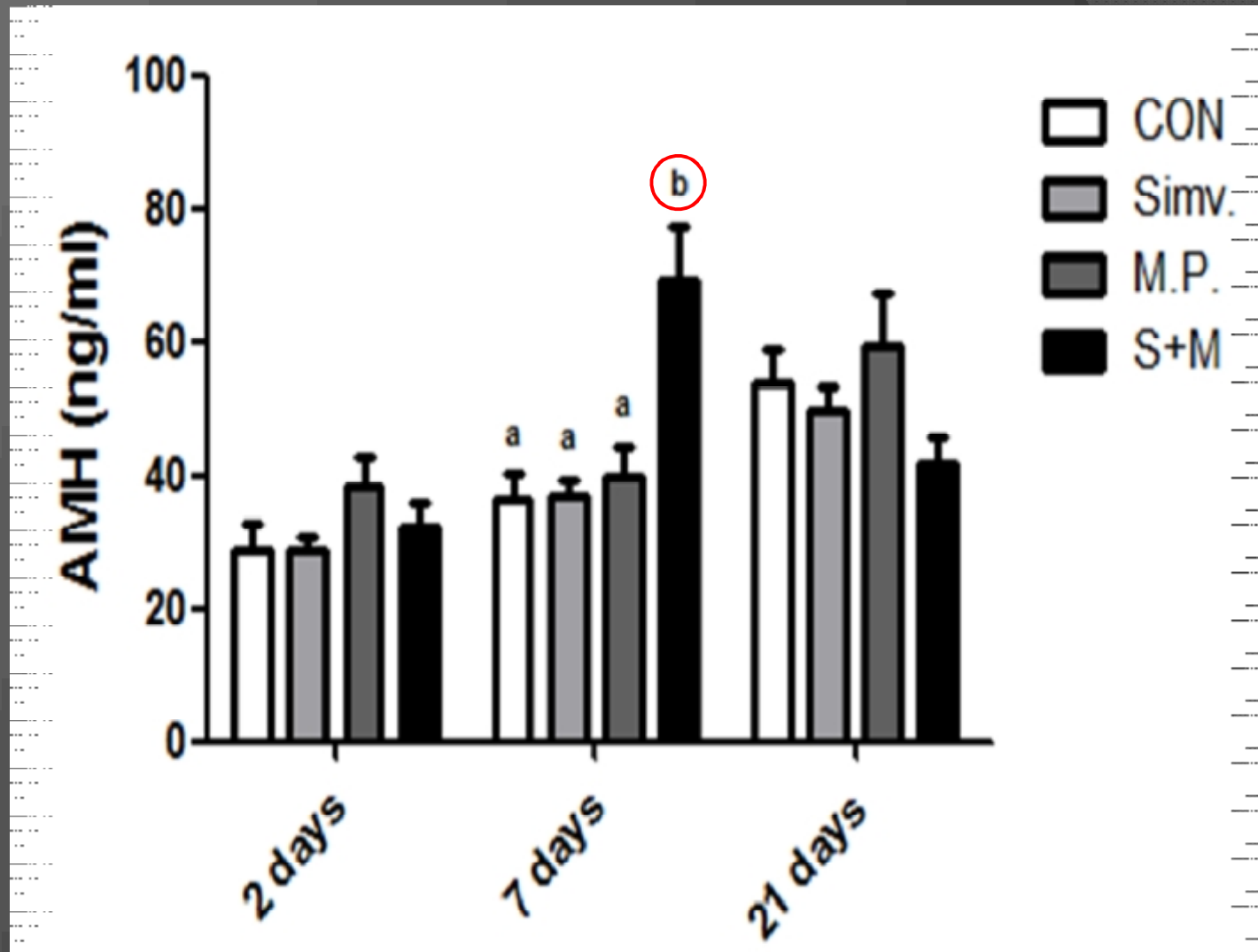


The Proportion of CD45 Cells



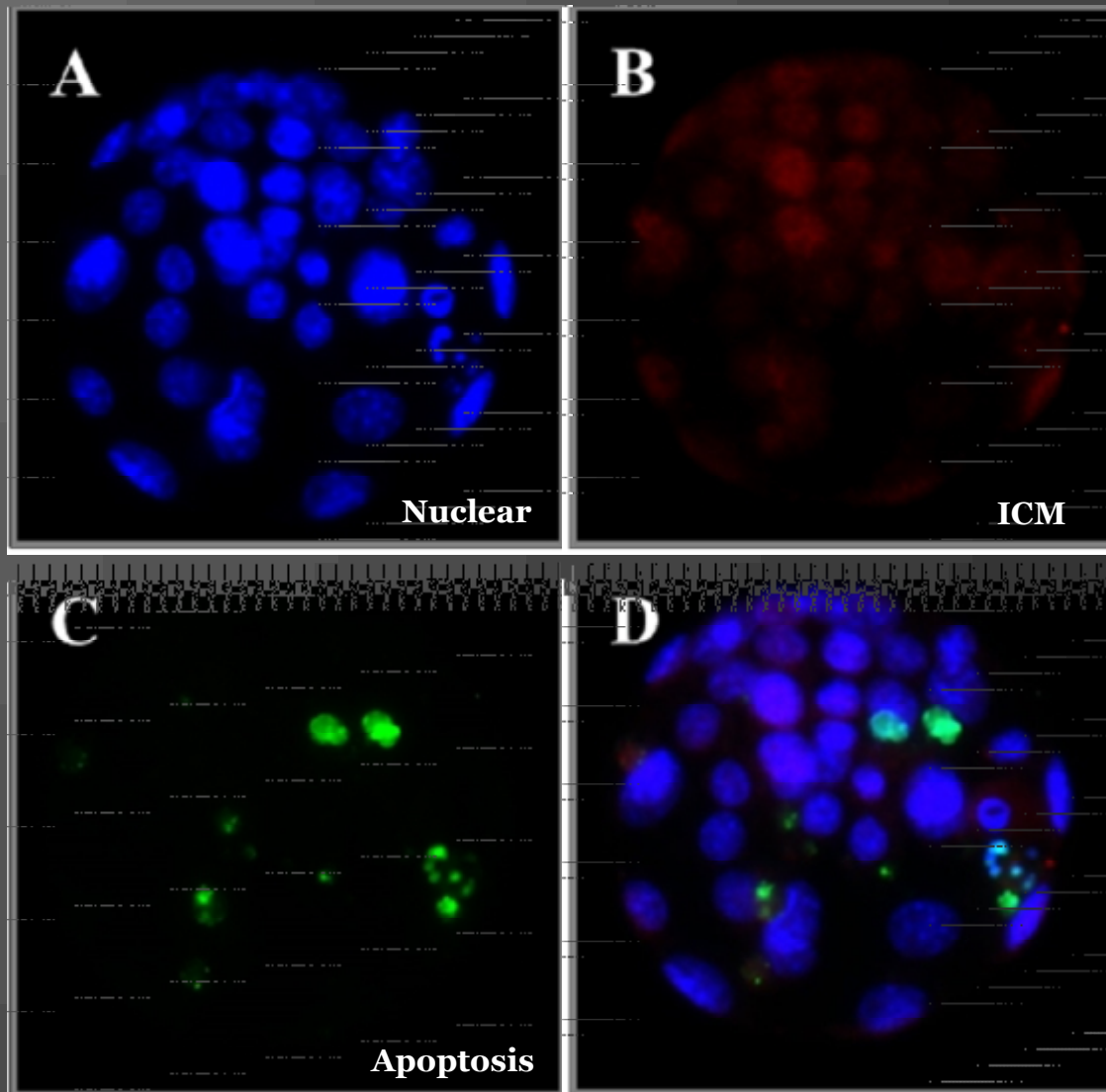
CD45: Leukocyte common antigen (LCA) De Vitro et al., Int J Mol Med (2012)

Serum AMH Levels



Anti-Mullerian Hormone (AMH): A biomarker for ovarian reserve

Differential Staining



A: Hoechst33342
B: Anti-Oct4
C: TUNEL
D: Merged

Embryonic Development after IVF

1147210123456789101112131415161718192021222324252627282930313233343536373839404142434445464748495051525354555657585960616263646566676869707172737475767778798081828384858687888990919293949596979899100

1147210123456789101112131415161718192021222324252627282930313233343536373839404142434445464748495051525354555657585960616263646566676869707172737475767778798081828384858687888990919293949596979899100



1147210123456789101112131415161718192021222324252627282930313233343536373839404142434445464748495051525354555657585960616263646566676869707172737475767778798081828384858687888990919293949596979899100



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1147210123456789101112131415161718192021222324252627282930313233343536373839404142434445464748495051525354555657585960616263646566676869707172737475767778798081828384858687888990919293949596979899100

ICM/TE (ea)	7.9±1.9/ 21.5±1.7	7.3±2.6/ 23.4±2.7	7.9±1.9/ 25.4±2.6	9.1±2.4/ 24.9±2.5
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1147210123456789101112131415161718192021222324252627282930313233343536373839404142434445464748495051525354555657585960616263646566676869707172737475767778798081828384858687888990919293949596979899100

Conclusion

- Treatment with combined Simvastatin and Methylprednisolone enhances the quality of ovarian tissues and reserve quantity.
 - ✓ **Primordial G₁**
 - ✓ **Apoptotic Follicle**
 - ✓ **CD31-positive area**
 - ✓ **Serum AMH**
- Although we evaluated the beneficial effects of Simvastatin and Methylprednisolone in the present study, we **could not unravel** the corresponding **protective mechanisms**
- This combinatorial treatment can be **clinically applied** to **humans** and **domestic animals** subject to further studies.