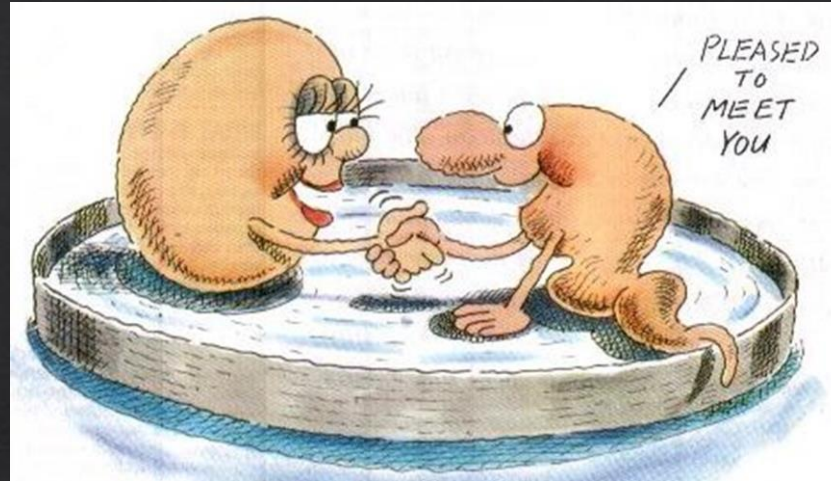


MED316
REPRODUCTIVE SYSTEM AND
DISORDERS



Assisted Reproduction Techniques

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Ankara University Faculty of Medicine
Dept. of Histology – Embryology
Lab Director - Center for Assisted Reproduction

Terms

- ◇ Assisted Reproduction Techniques (ART)
- ◇ Intrauterine Insemination (IUI)
- ◇ In-vitro fertilization (IVF)
- ◇ Intracytoplasmic sperm injection (ICSI) – Microinjection
- ◇ Cryopreservation
- ◇ Oocyte + spermatozoon = Gametes
- ◇ Ovarium + testis = Gonads
- ◇ Zygote, embryo, blastocyst, fetus (week 11)
- ◇ Fertility
- ◇ Infertility / Sterility
- ◇ Pre-implantation genetic test (PGT)



Aims and goals

- ◆ The goal of Assisted Reproductive Techniques is to help an infertile couple, take a single and healthy baby home.
- ◆ Take home baby rates
- ◆ Pregnancy rates
- ◆ Blastocyst growth rates
- ◆ Fertilization rates




Indications

◇ Infertility

- ◇ It is defined as the inability of a couple who has sexual intercourse without protection to have a child despite trying for a year.

◇ Fertility preservation

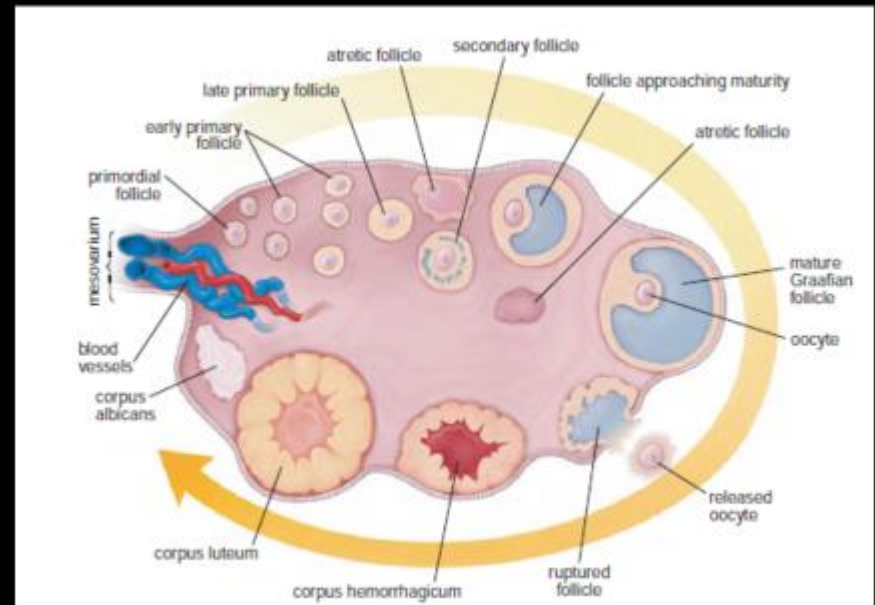
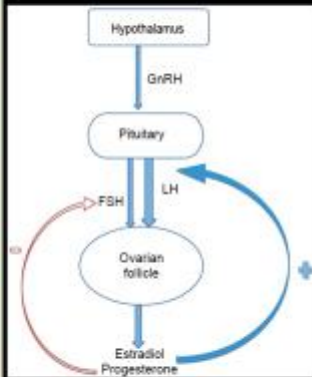
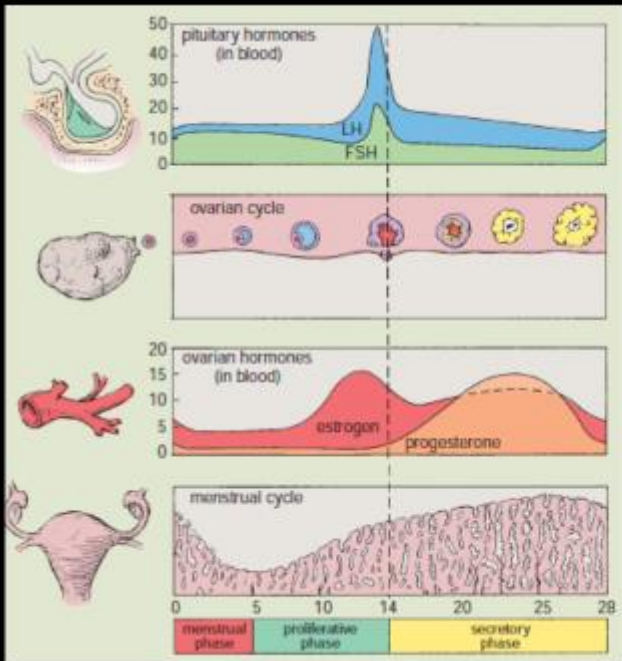
- ◇ Freezing the gametes or gonads of individuals who have a high risk of experiencing infertility in their future life for any reason by storing them in liquid nitrogen until needed.
 - ◇ Before cancer chemo/radiotherapy, in diseases with decreased gonadal reserve (Turner Syndrome, premature ovarian failure, etc.)
- ## ◇ Birth of a child suitable for stem cell donation for a sibling



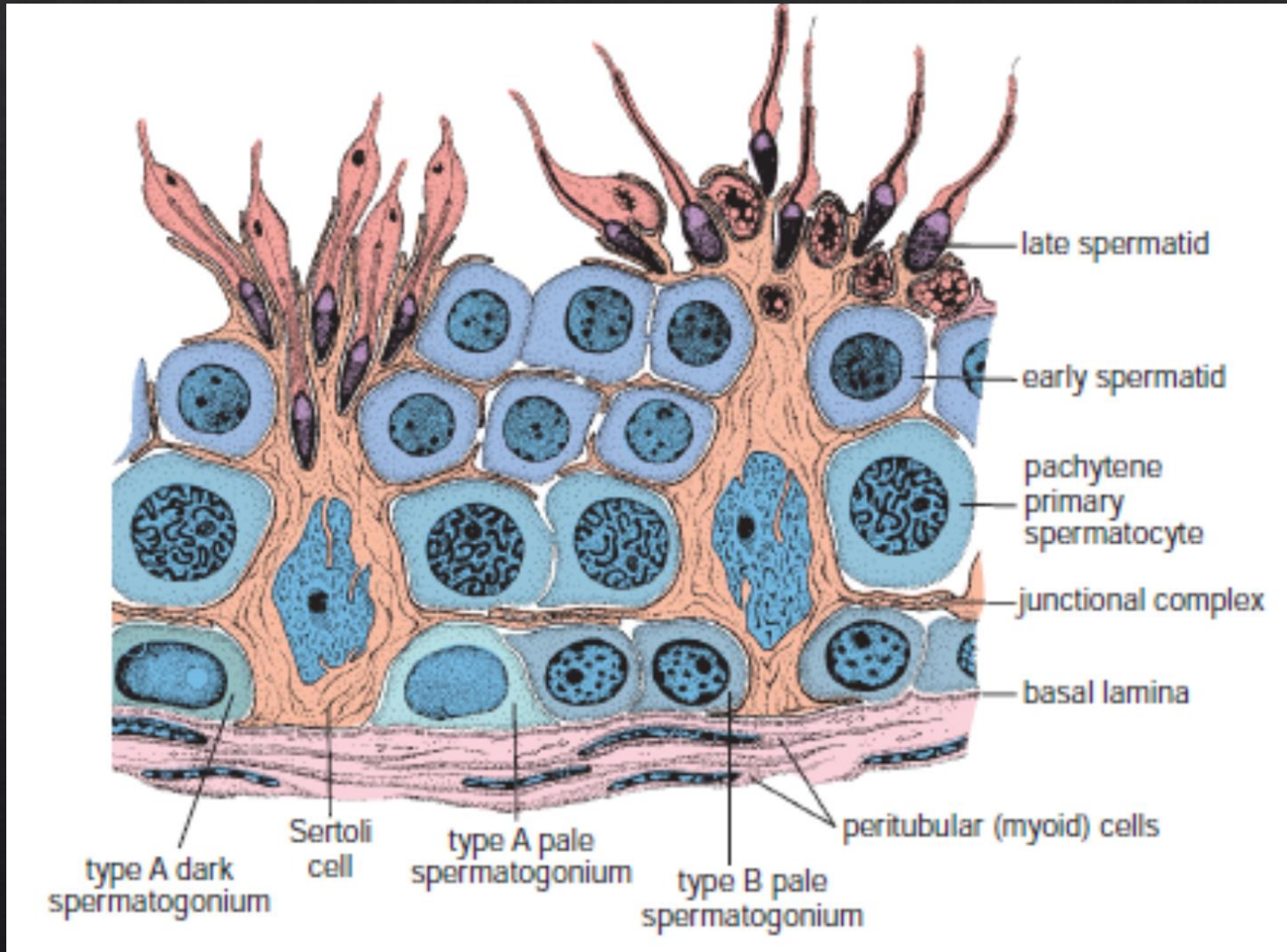
Reproduction is essential universally...

- ◇ **Individuals' instincts/rights to start a family and to reproduce**
- ◇ **Continuity of life (population)**
- ◇ **Transfer of your genes and personal characteristics to new generations**
- ◇ **The emergence of different genes, diversity**

Oogenesis



Spermatogenesis



www.7activemedical.com

During normal fertilization



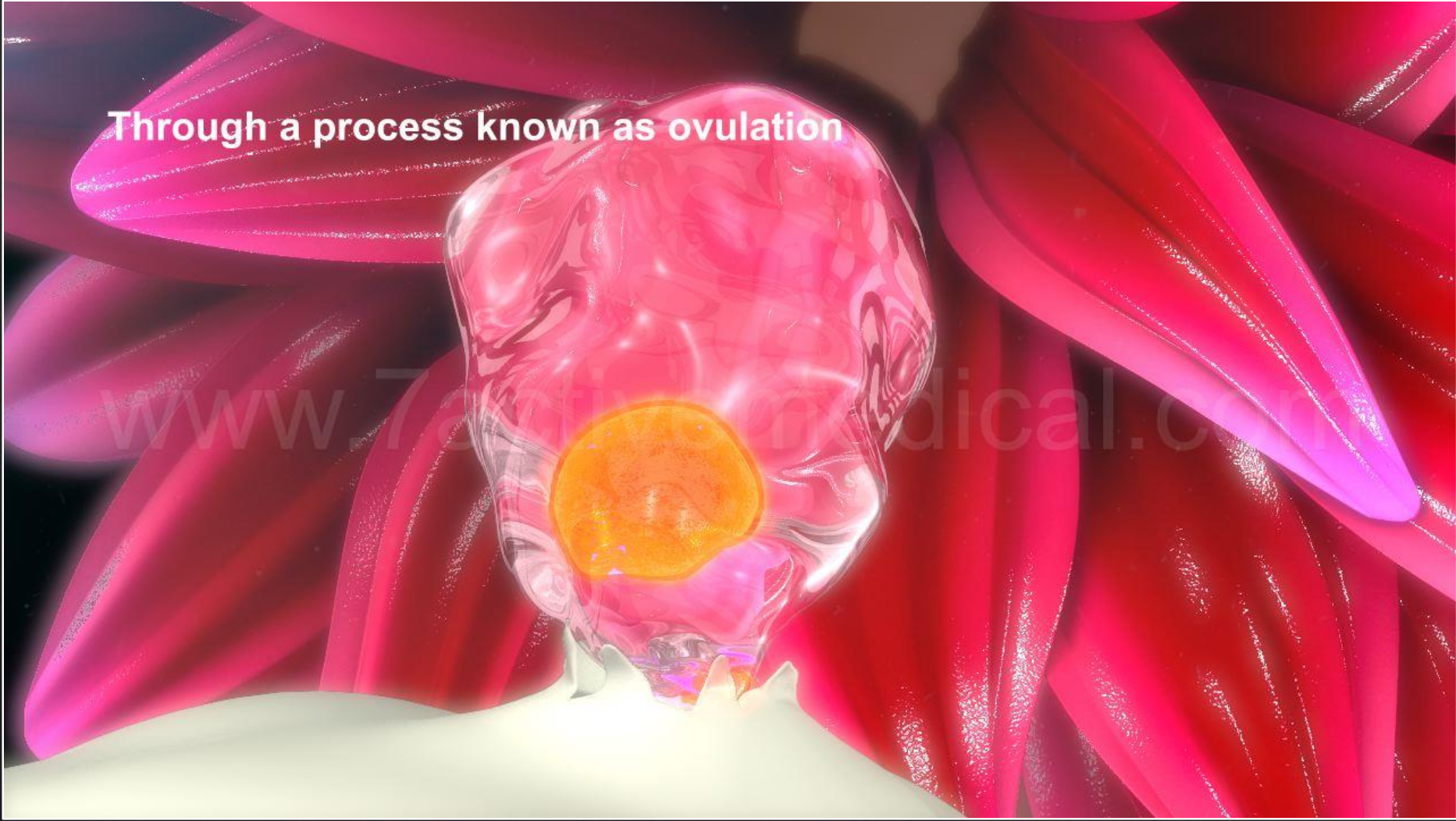
A mature egg is released by the ovary

www.7actical.com



Through a process known as ovulation

www.fertilitymedical.com






Which is then picked up by the fallopian tube

A close-up photograph of a dog's face, focusing on its nose. The dog's fur is a mix of brown and black. A bright, glowing yellow circle is superimposed on the bridge of its nose. The background is dark and out of focus. A faint watermark 'medical.com' is visible across the middle of the image.

En route to the uterus



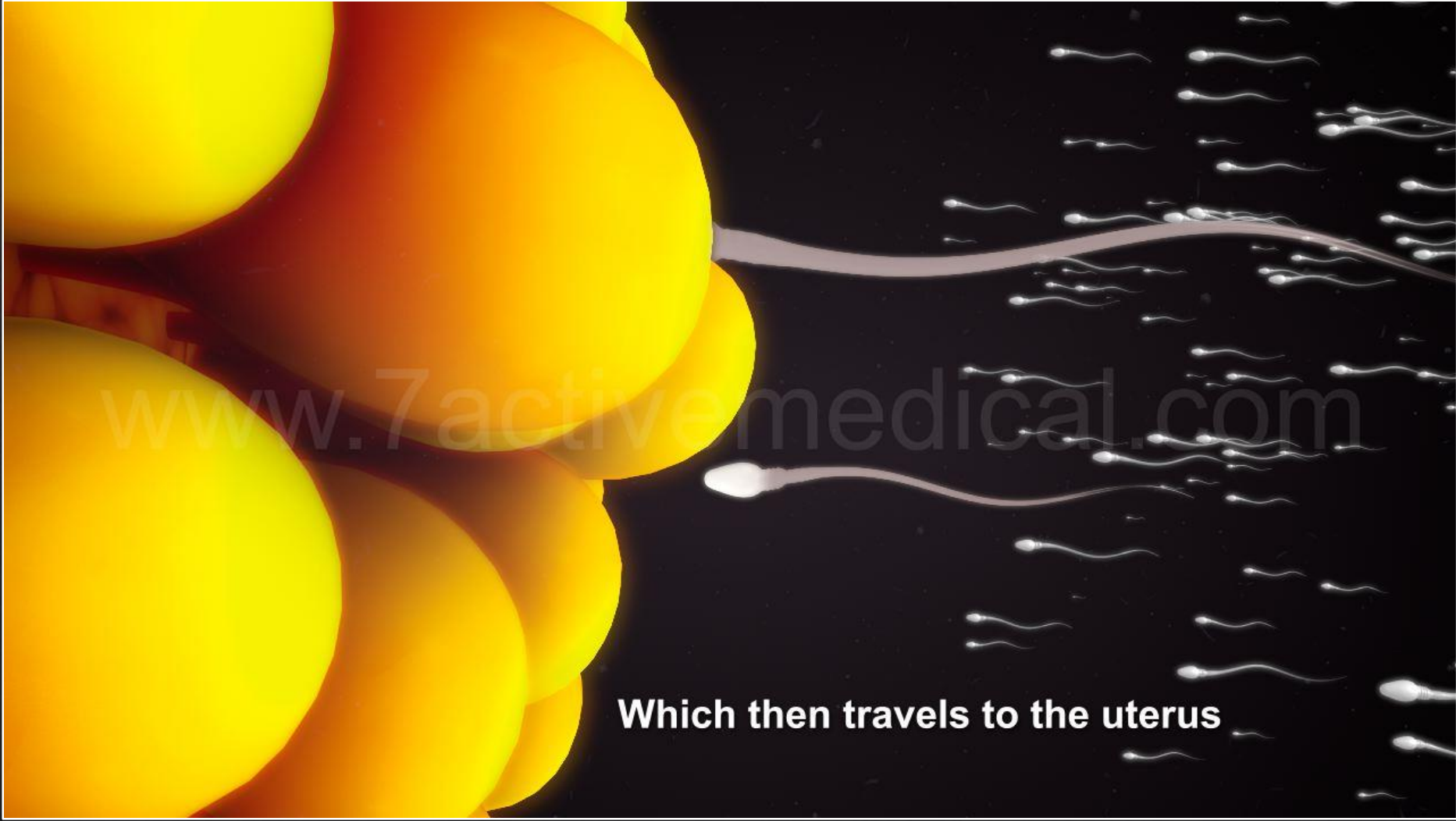
A woman's chances of conceiving, are best, before during or immediately after ovulation takes place.

A 3D illustration of a sperm cell cluster and individual sperm cells. The central focus is a large, spherical cluster of numerous small, bright yellow spheres, representing a sperm cell or a cluster of sperm cells. Surrounding this central cluster are several individual sperm cells, each depicted as a small, white, teardrop-shaped head with a long, thin, wavy tail. The background is a dark, almost black, space with a subtle, grainy texture. A faint watermark, "www.7a...cal.com", is visible across the middle of the image.

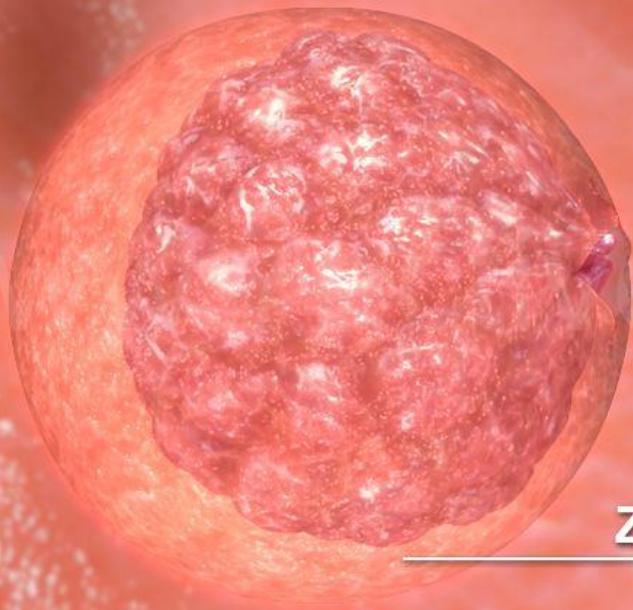
Sperm cells from the man's semen



Fertilize the egg inside the fallopian tube



Which then travels to the uterus



Zona pellucida

In the uterus, the egg loses its outer protective covering, known as the zona pellucida



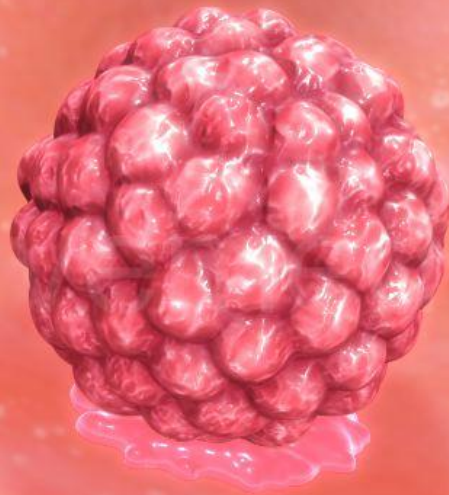
Zona hatching

Through a process known as zona hatching



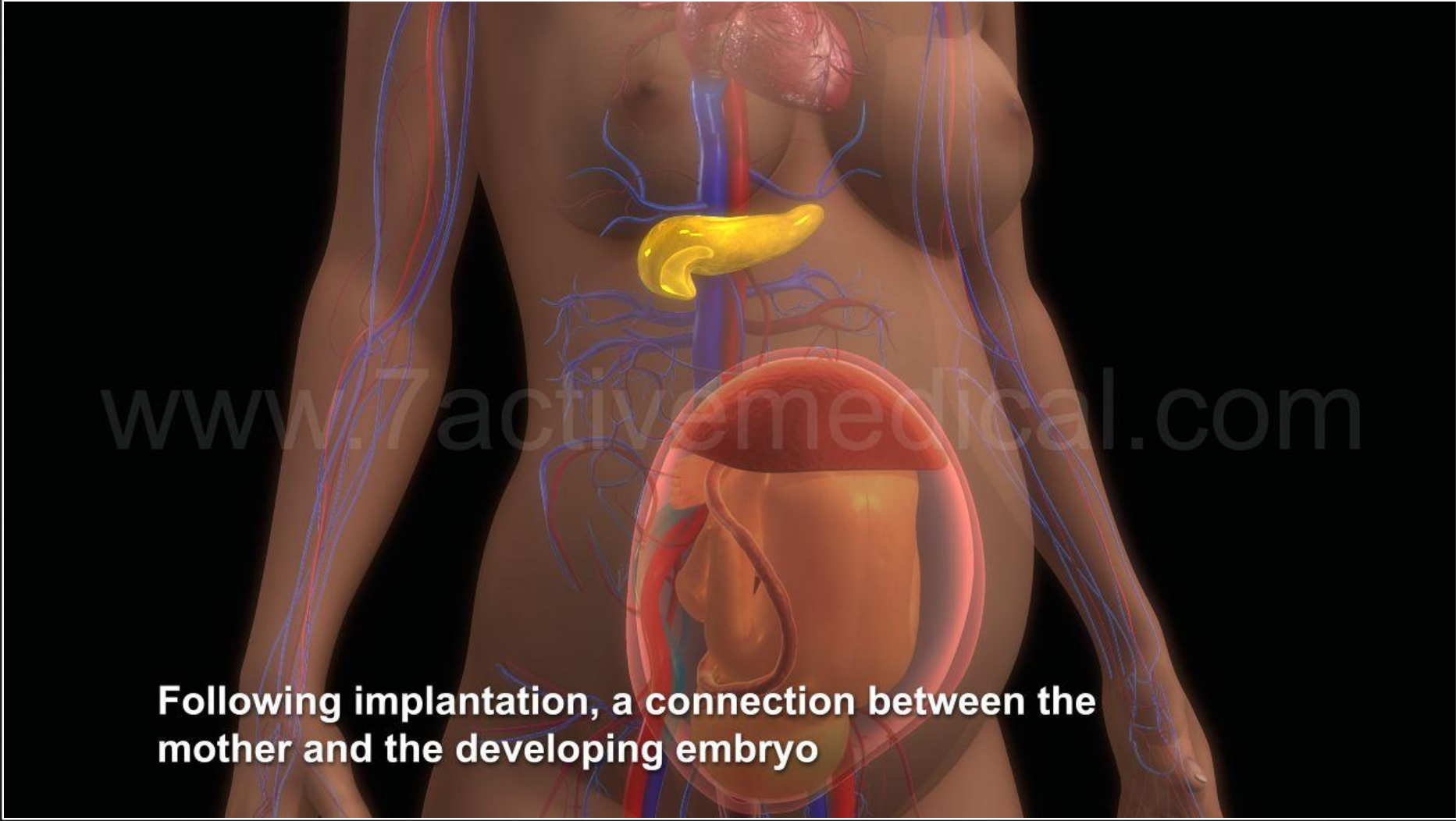
Implantation

Which is important for the implantation of the fertilized egg

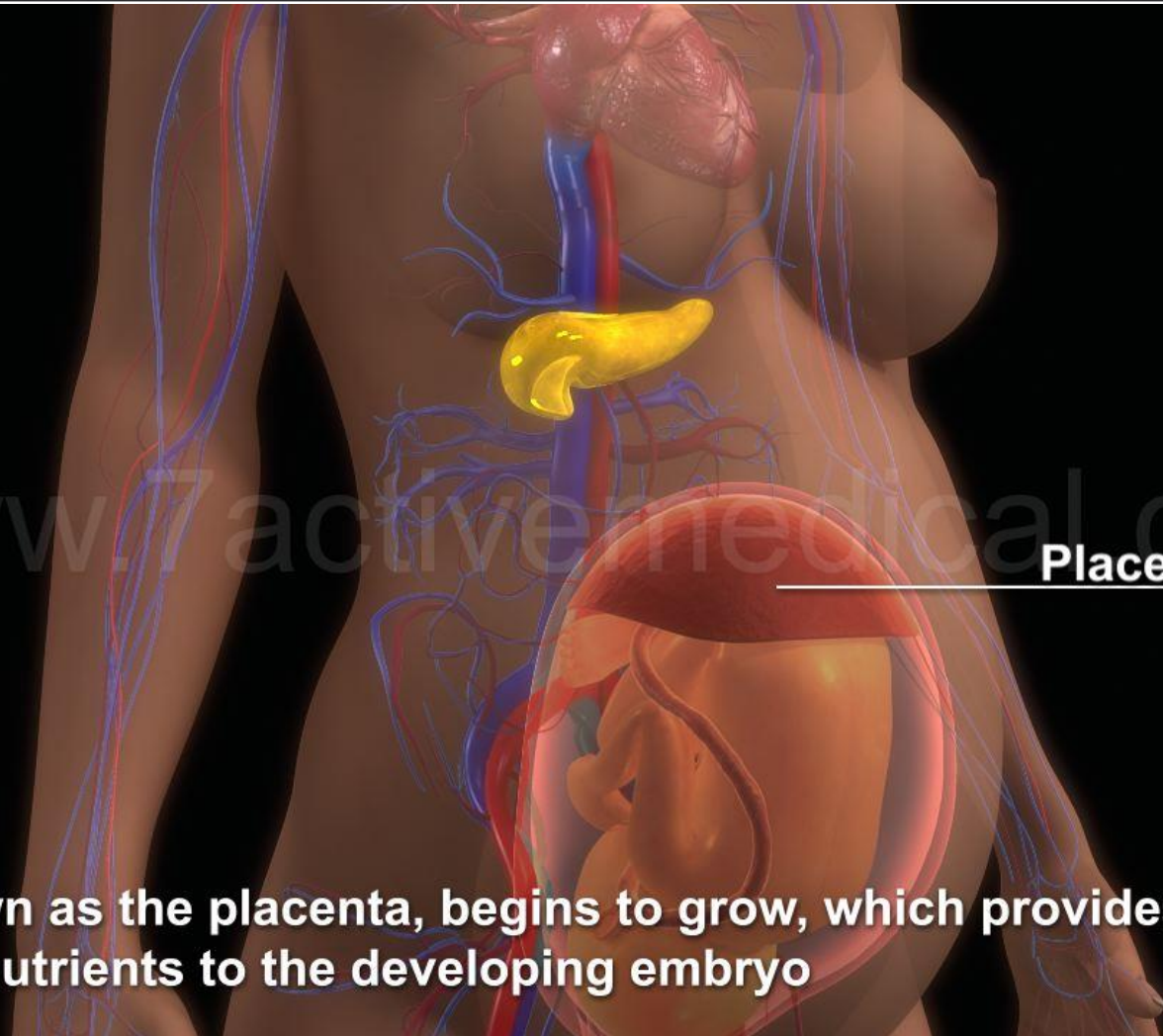


www.7active.com

Onto the endometrial lining of the uterus



Following implantation, a connection between the mother and the developing embryo



Placenta

Known as the placenta, begins to grow, which provides oxygen and nutrients to the developing embryo

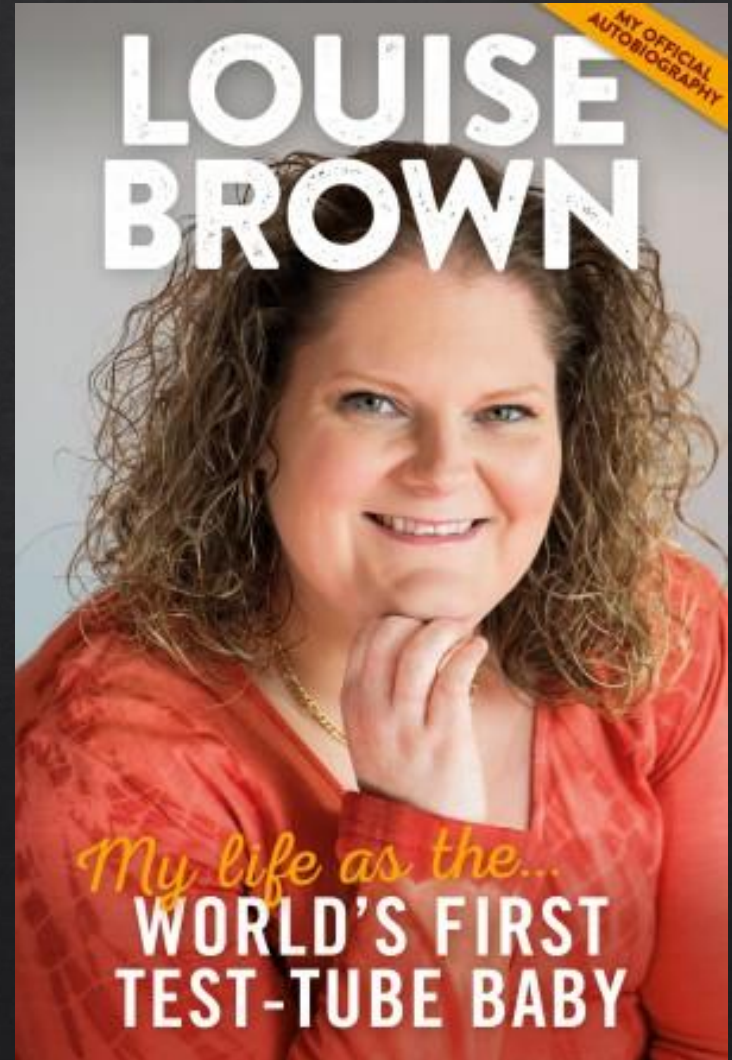
HISTORY OF IVF

Louise Joy Brown



June 25th, 1978
Oldham-England

Louise Joy Brown



2010 Nobel Prize in Physiology
«*the development of in-vitro fertilisation*»



Robert G. Edwards
1925 - 2013



Patrick Steptoe
1913 - 1988





1958 – Carl Gemzell

Obtaining the first pregnancy by giving hormones externally
(Sweden)

1959 – M.C. Chang

The first rabbit embryos obtained by
in-vitro fertilization and live birth
(USA)





1961 – Palmer

Taking oocytes out of the body for the first time
with laparoscopy (France)

1973 – Carl Wood and John Leeton

Achievment of the first IVF pregnancy
Resulted in early pregnancy loss
(Australia)



He who laughs last laughs longest...





1992 - Gianpiero D. Palermo

First Birth After Intrastoplasmic Sperm Injection
(Belgium)

June 23, 1988 - the first IVF center in Turkey was established at Ege University with the efforts of [Prof. Refik Çapanoğlu](#) and his colleagues.

Turkey's first test-tube baby was born on April 18, 1989 at this center.



Ece okar

Turkey's first test-tube baby is from Harvard

Turkey's first test-tube baby born on April 18, 1989, Ece okar is now 27 years old. Ece, studied Law at Harvard, now works as a lawyer with her mother.



The very first case



Lesley and John Brown

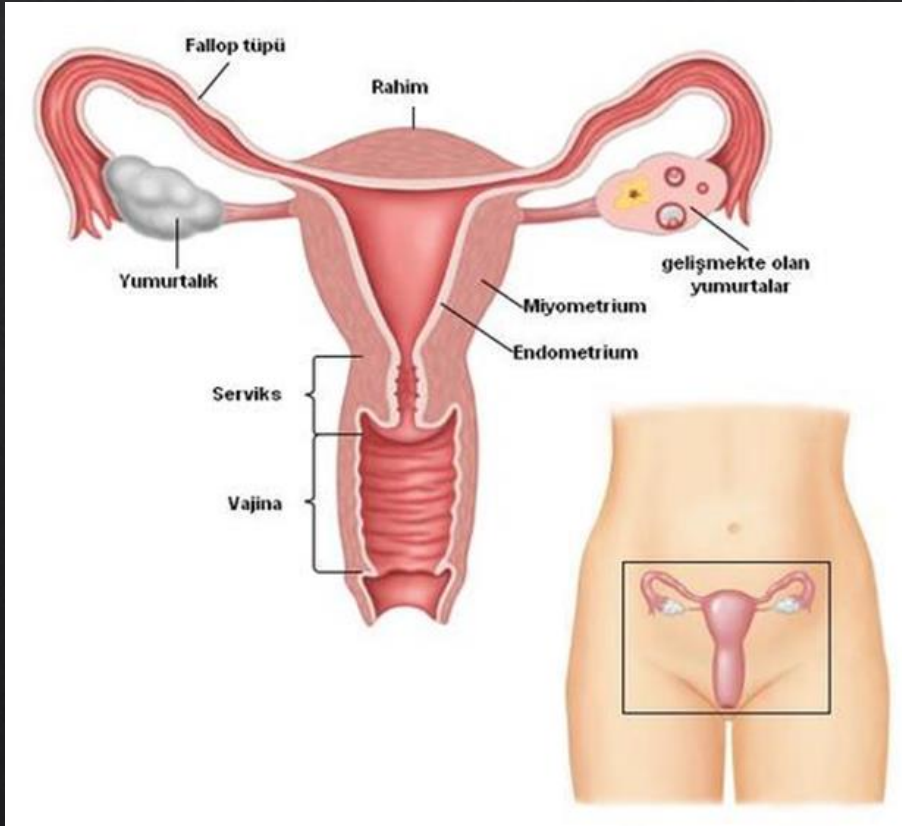
They were unable to have a baby
despite nine years

Lesley Brown's Fallopian tubes were
blocked

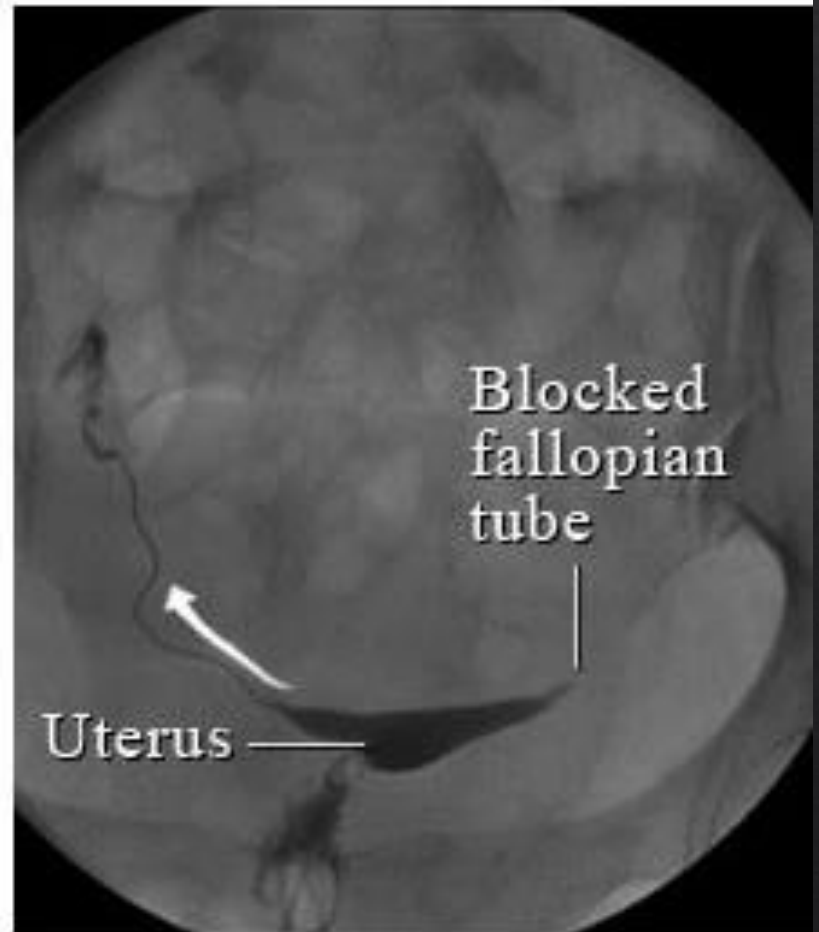
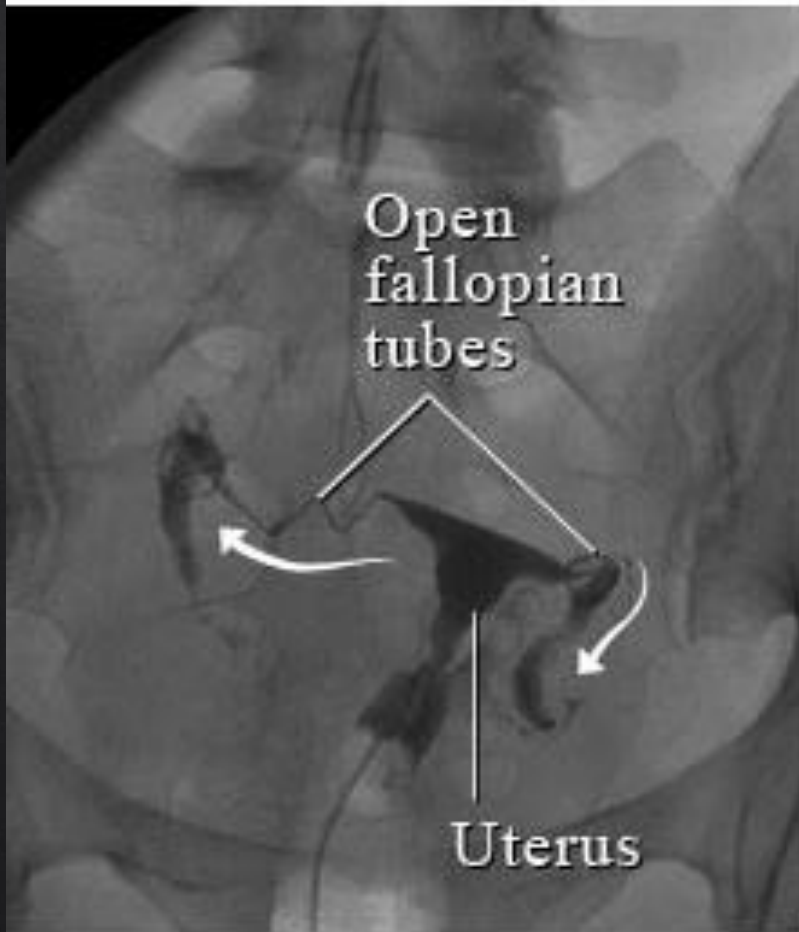
IVF was applied on November 10,
1977



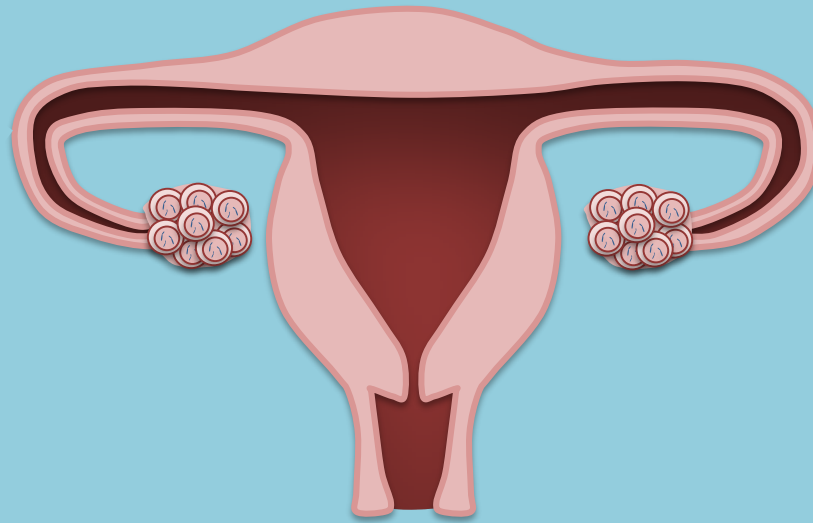
Hysterosalpingography (HSG)



Tubal Factor

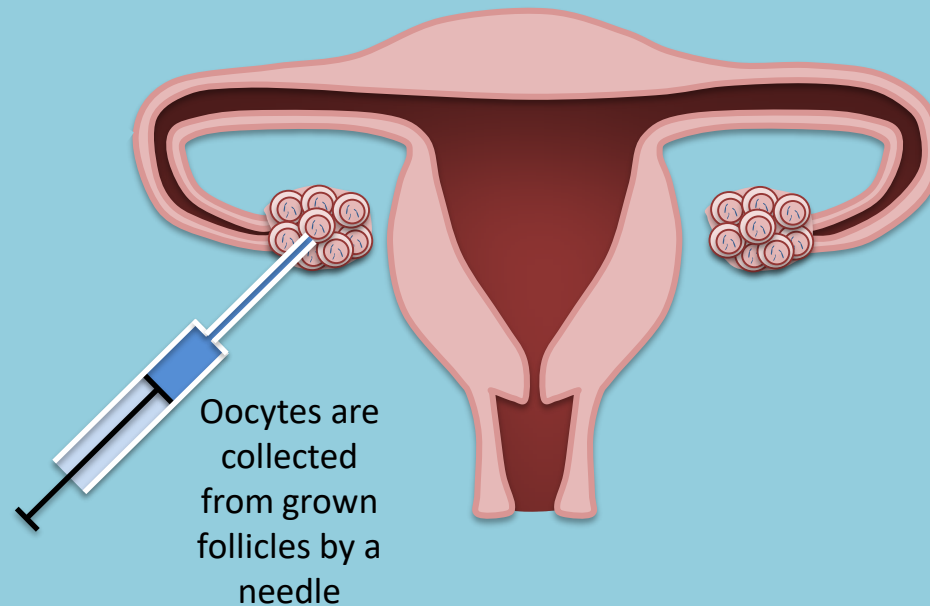


By giving hormones externally and in supraphysiological doses, oocytes are allowed to develop more than normal in numbers (10-15 days)

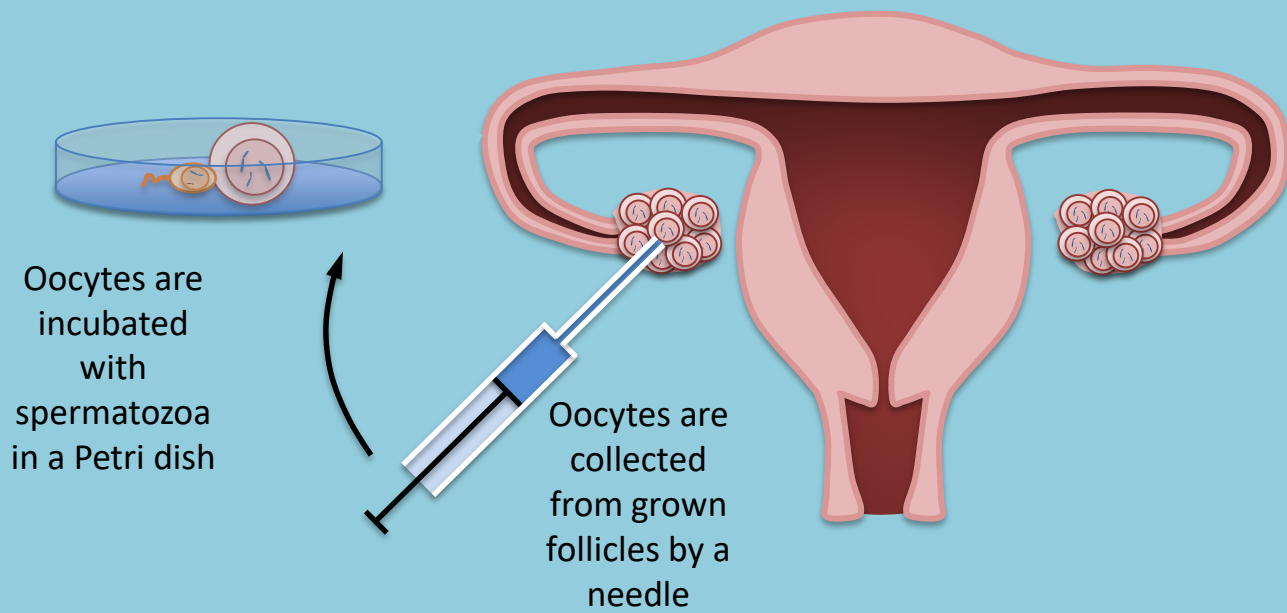


IVF-oocyte pick-up

By giving hormones externally and in supraphysiological doses, oocytes are allowed to develop more than normal in numbers (10-15 days)

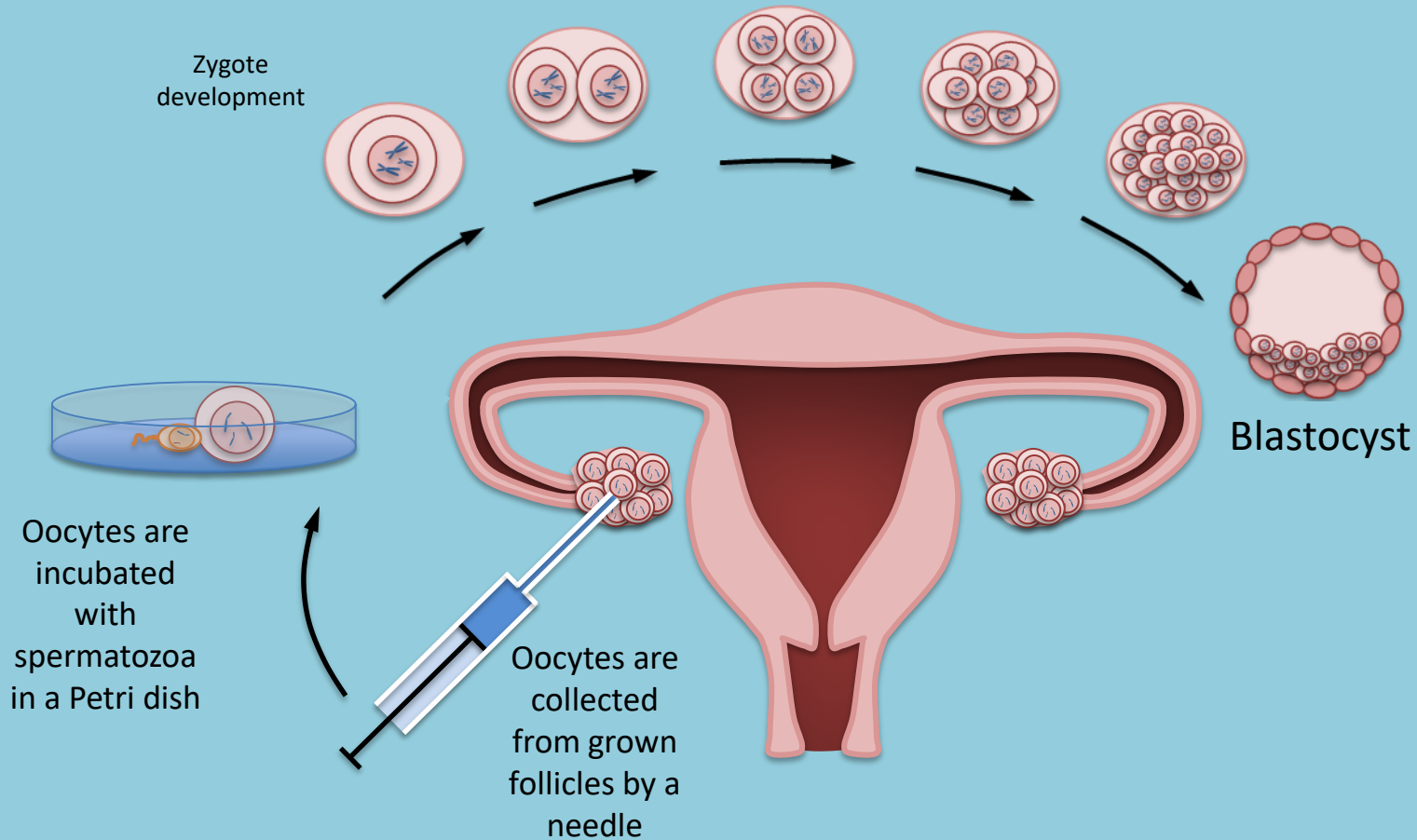


IVF-oocyte pick-up



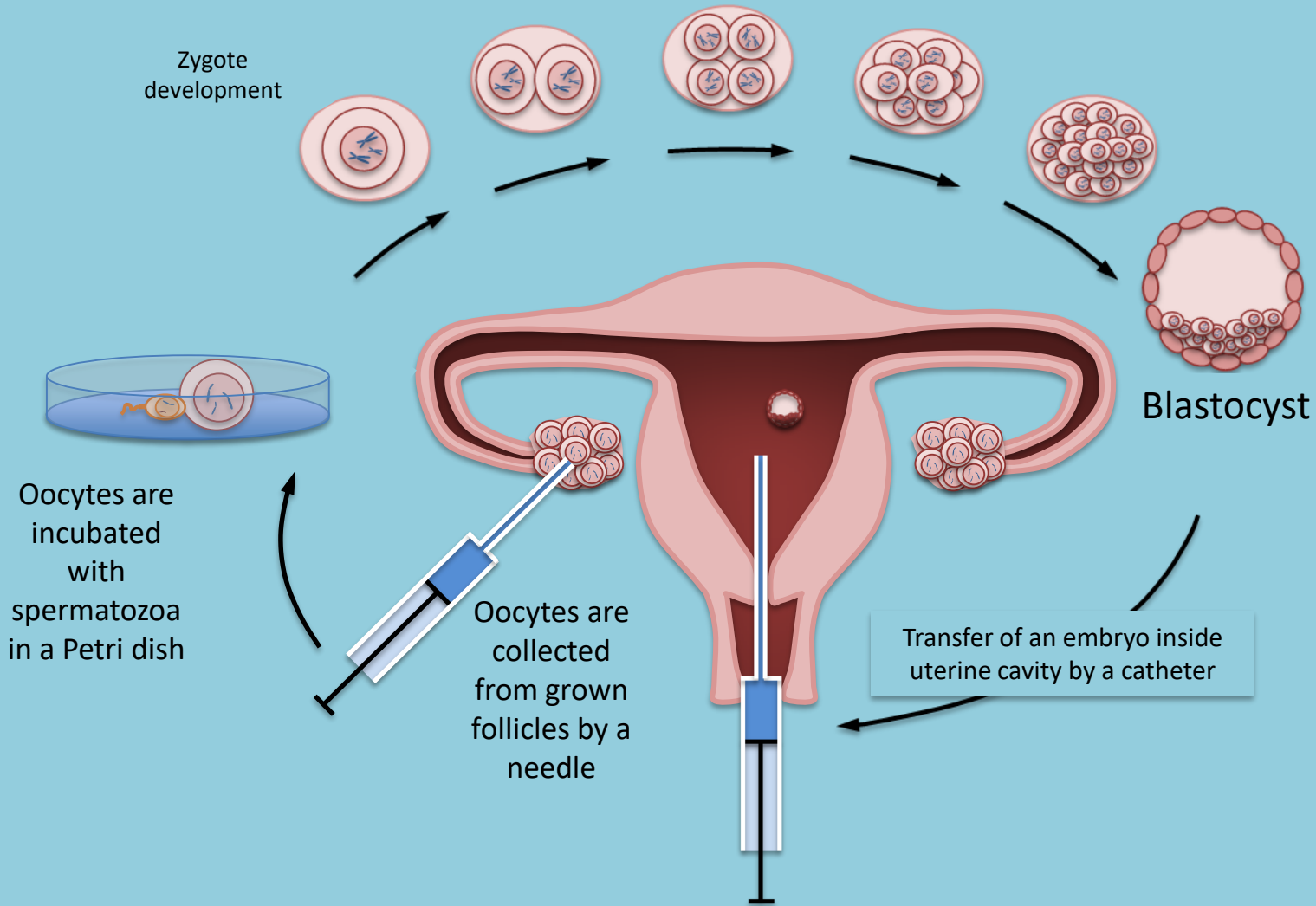
IVF-classical IVF

In-vitro culture of the embryos for 2 to 5 days



IVF-embryo culture

In-vitro culture of the embryos for 2 to 5 days



IVF-embryo transfer



1978



1978



Natalie Brown – 1982
40th baby born with IVF



1978



Natalie Brown – 1982
40th baby born with IVF



Natalie Brown –
first person who
conceived with
IVF and gave
spontaneous
birth (1999)
(Natural
pregnancy)



1978

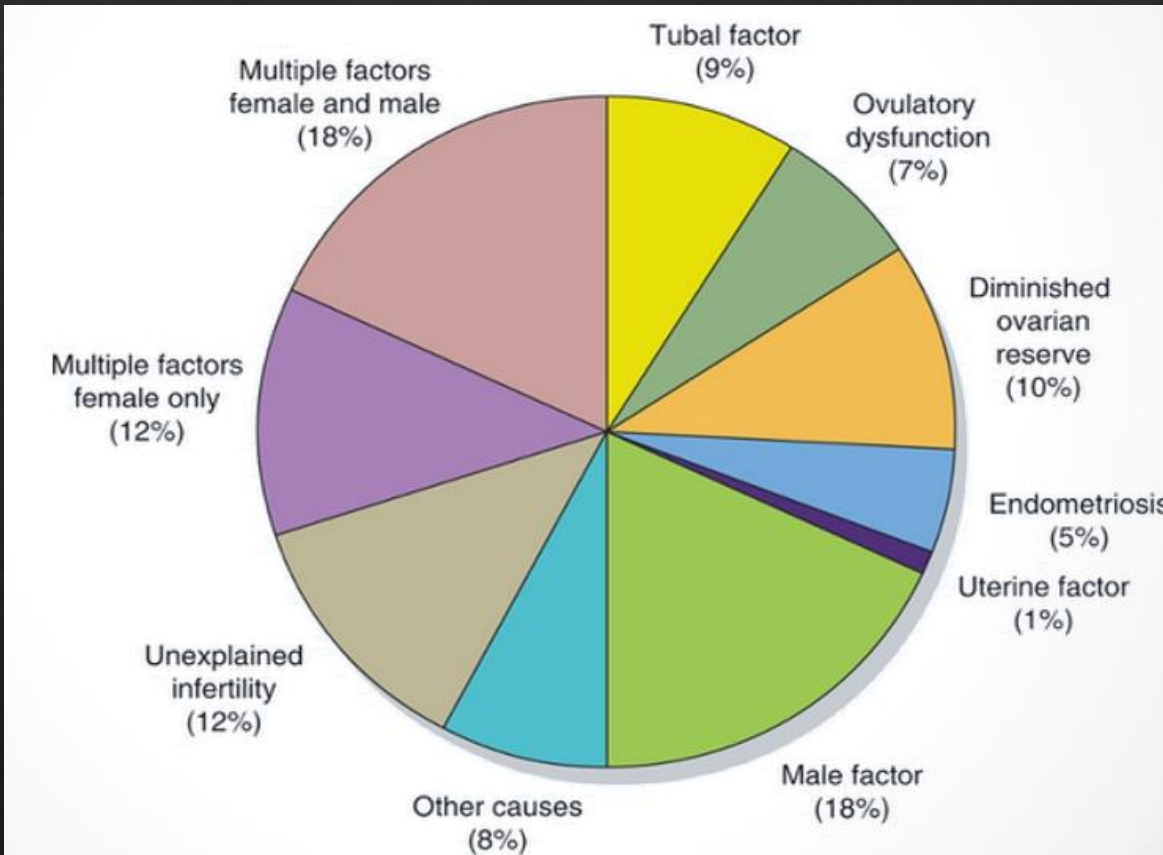


Natalie Brown – 1982
40th baby born with IVF



Natalie Brown –
first person who
conceived with
IVF and gave
spontaneous
birth (1999)
(Natural
pregnancy)

IVF INDICATIONS



Female: %35
Male : %35
Both: %18
Unexplained: %12

Male-related factors

◇ Endocrine Disorders

- ◇ Hypothalamic dysfunction (Kallmann Syn.)
- ◇ Pituitary insufficiency (tumor, radiation, surgery)
- ◇ Hyperprolactinemia, adrenal hyperplasia
- ◇ Exogenous use of androgens, thyroid diseases

◇ Anatomical disorders

- ◇ Congenital absence of the vas deference
- ◇ Vas deference obstruction
- ◇ Congenital anomalies of the ejaculatory system

Male-related factors

◇ Abnormal Spermatogenesis

- ◇ Chromosomal abnormalities
- ◇ Mumps orchitis, Cryptorchidism, Varicocele
- ◇ Drug or radiation exposure

◇ Abnormal Motility

- ◇ Absence of cilia (Kartegener syndrome)
- ◇ Varicocele, Antisperm antibody

◇ Sexual Dysfunction

- ◇ Retrograde ejaculation, Impotence (ED), Libido loss

Female-related factors

◇ Tubal factor

- ◇ Bilateral obstruction of the Fallopian tubes

◇ Anatomical disorders

- ◇ Uterine anomalies, developmental disorders
- ◇ Presence of a myoma


◇ Ovulatory disorders

- ◇ Oocyte development is present but cannot be ovulated from the ovary - Polycystic ovary syndrome

◇ Endometriosis

SART

← → ↻ 🏠 Güvenli | <https://www.sartcorsonline.com/Predictor/Patient> ☆ Uygulamalar ★ Bookmarks Welcome to Facebook maps - Google'da Ara Favorilerim Google Maps Wikipedia, the free en Google Translate Home - PubMed - NC » | Diğer yer işaretleri



Society For Assisted
Reproductive Technology

What are my chances with ART?

While more models are coming soon, this model includes sequential transfers of fresh embryos only, no frozen transfers.

Welcome to the SART Patient Predictor. SART has developed this predictor based on nearly 500,000 cycles of therapy to more than 320,000 women throughout the United States since 2006^{1,2}. This calculator is meant to help you understand your chances of having a live birth, based on your personal situation. The answers to the following questions influence the likelihood that the IVF treatment will be successful. Some of the answers you may know — like your height and weight. Other answers — such as the diagnosis of the cause of infertility — will depend on whether or not you have been seen by a physician, and may change during your course of treatment. The calculations from this Patient Predictor assume that you have not had prior IVF treatment.

Background and Reproductive History

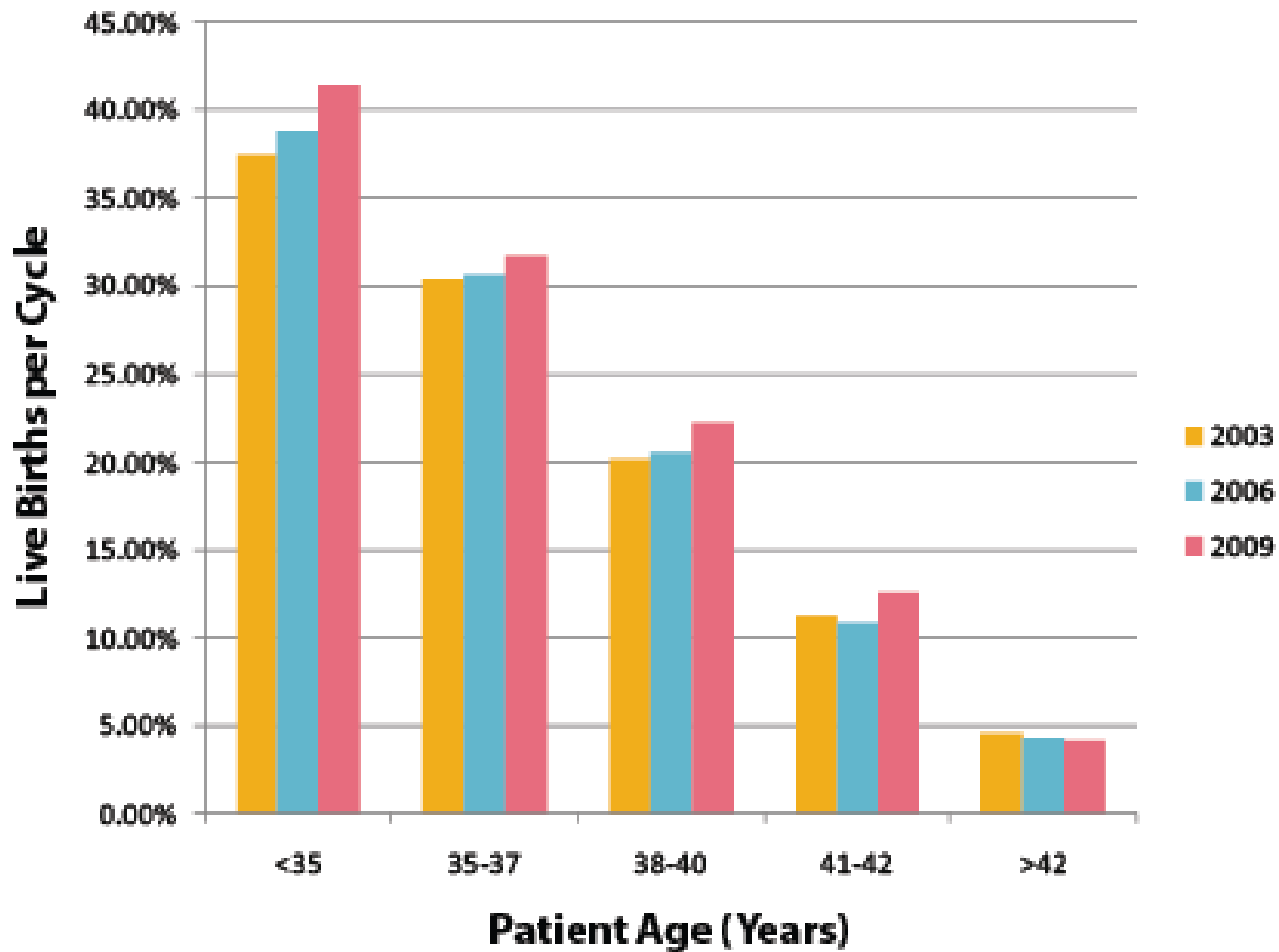
How old are you?

How tall are you?
 feet inches feet meters

How much do you weigh?
 lbs lbs kgs

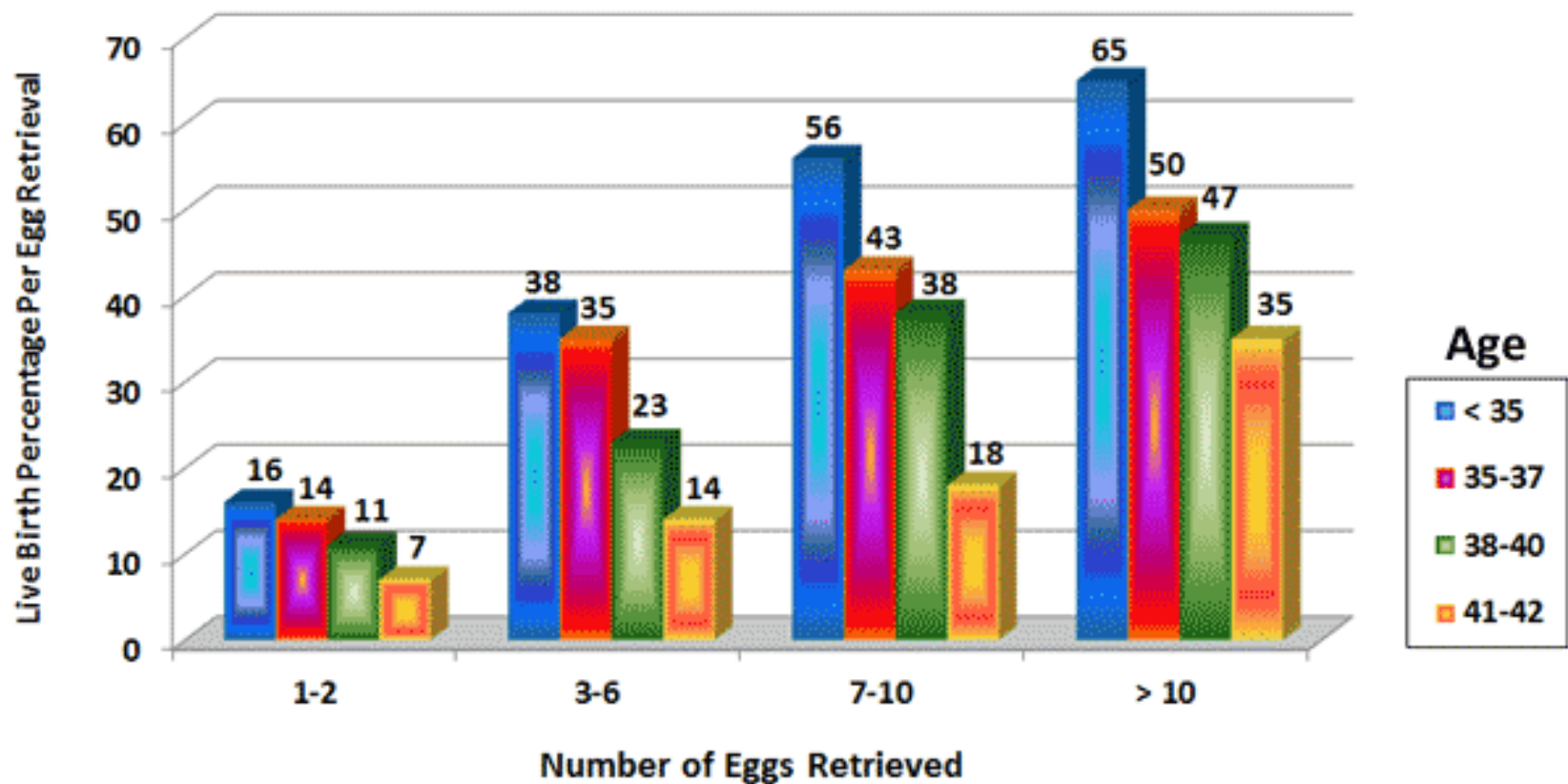
How many prior pregnancies have you had? ⓘ

How many prior full-term (>= 37 weeks) births have you had? ⓘ

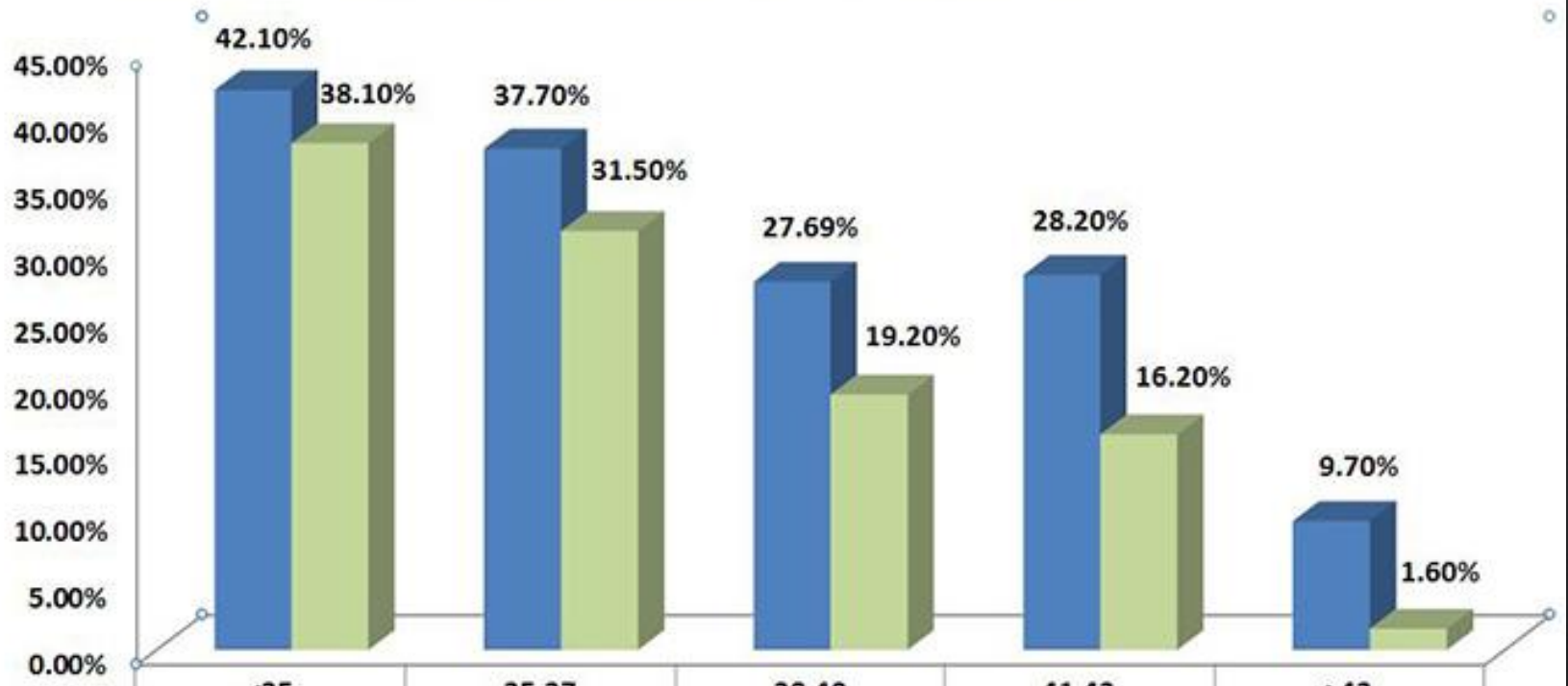


Live Birth Rate Per Egg Retrieval by Number of Eggs and Age

Jan 2001 - June 2009, 2043 Egg Retrievals



UCSF CLINICAL PREGNANCIES & LIVE BIRTH PER CYCLE IN 2012



■ % of Cycles resulting in Pregnancies 2012

■ % of Cycles resulting in Live Births 2012

<35
n = 126

35-37
n = 130

38-40
n = 156

41-42
n = 117

>42
n = 62

42.10%

37.70%

27.69%

28.20%

9.70%

38.10%

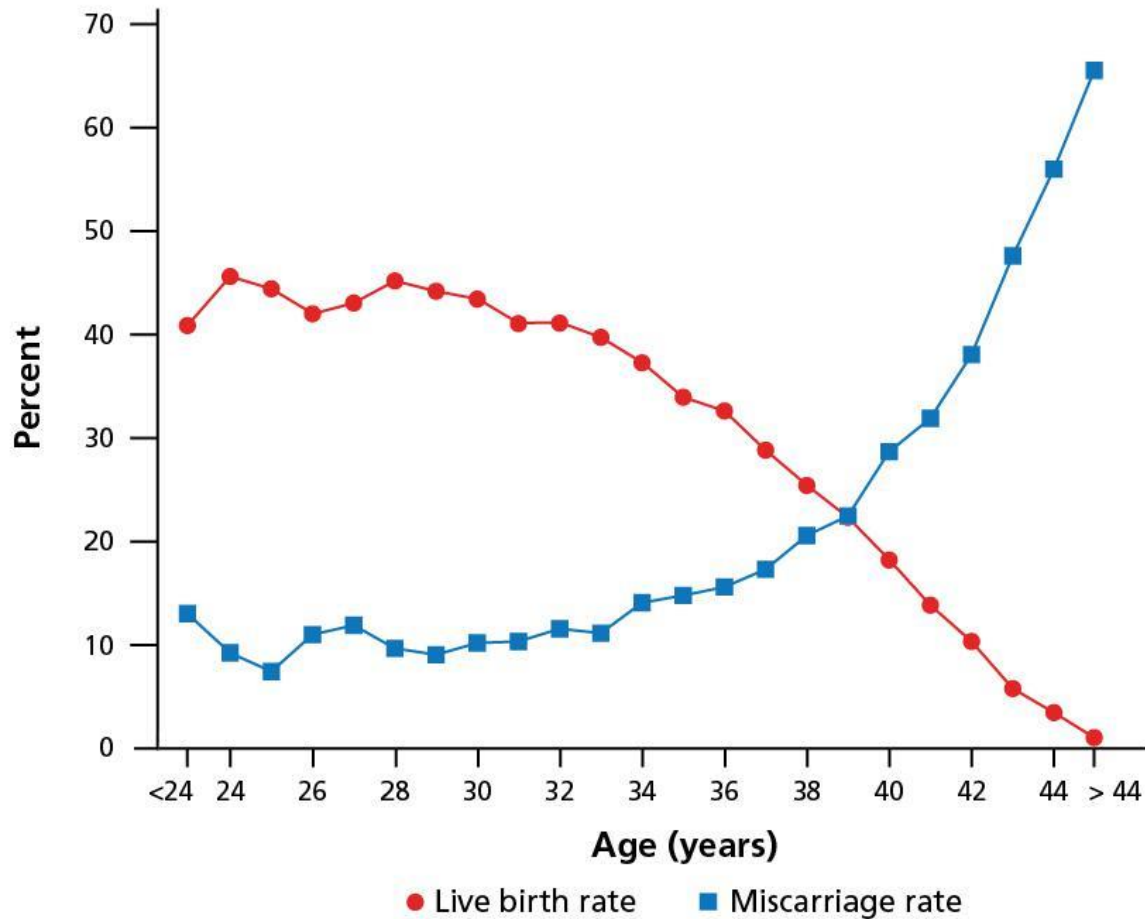
31.50%

19.20%

16.20%

1.60%

Percentages of ART Cycles Using Fresh Nondonor Eggs or Embryos That Resulted in Live Birth and Miscarriage, by Age of Woman (U.S. 2010).



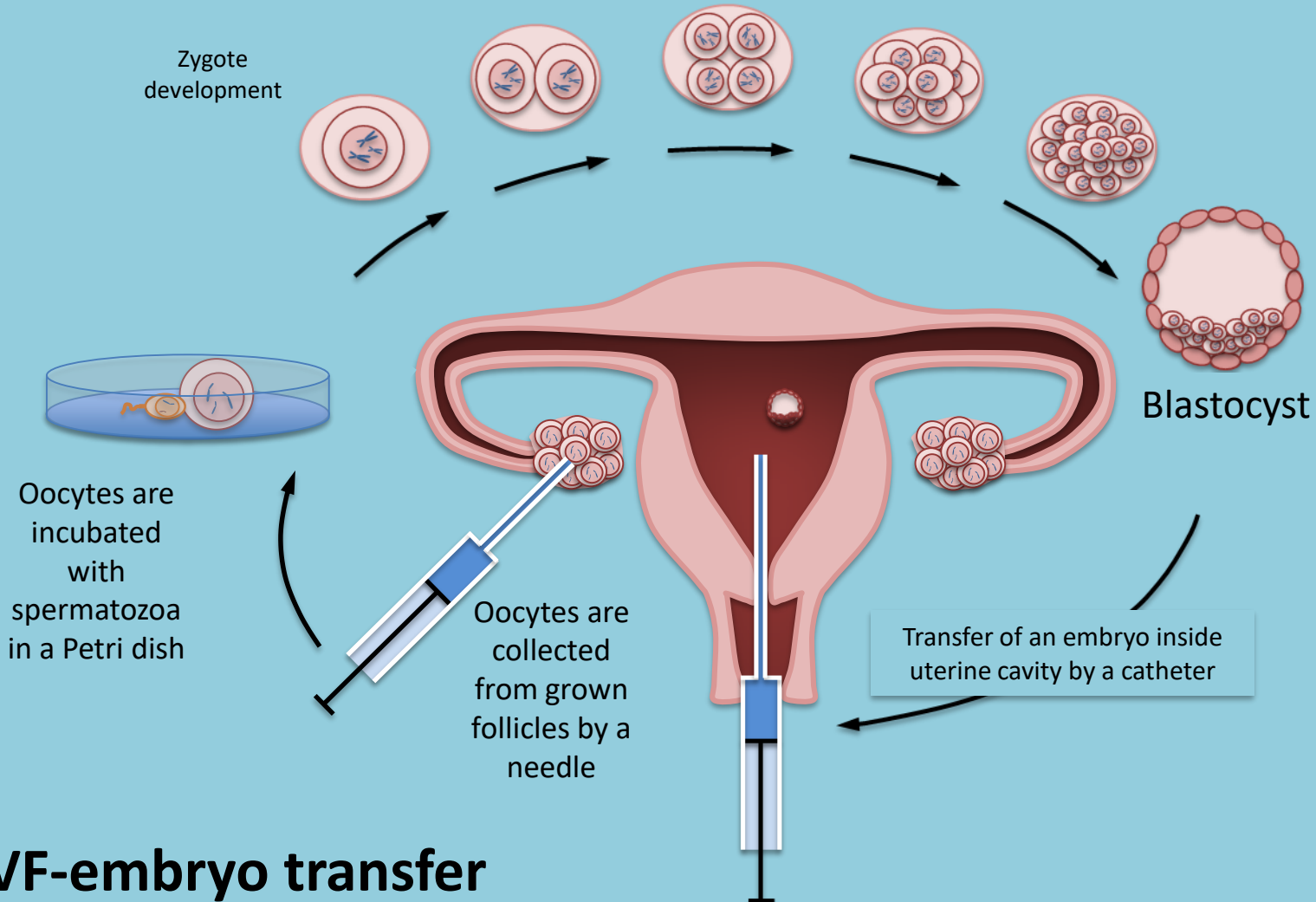
Adapted from: Centers for Disease Control and Prevention, American Society for Reproductive Medicine, Society for Assisted Reproductive Technology. 2010 *Assisted Reproductive Technology National Summary Report*. Atlanta: U.S. Department of Health and Human Services; 2012.

Make a child, a career too ...

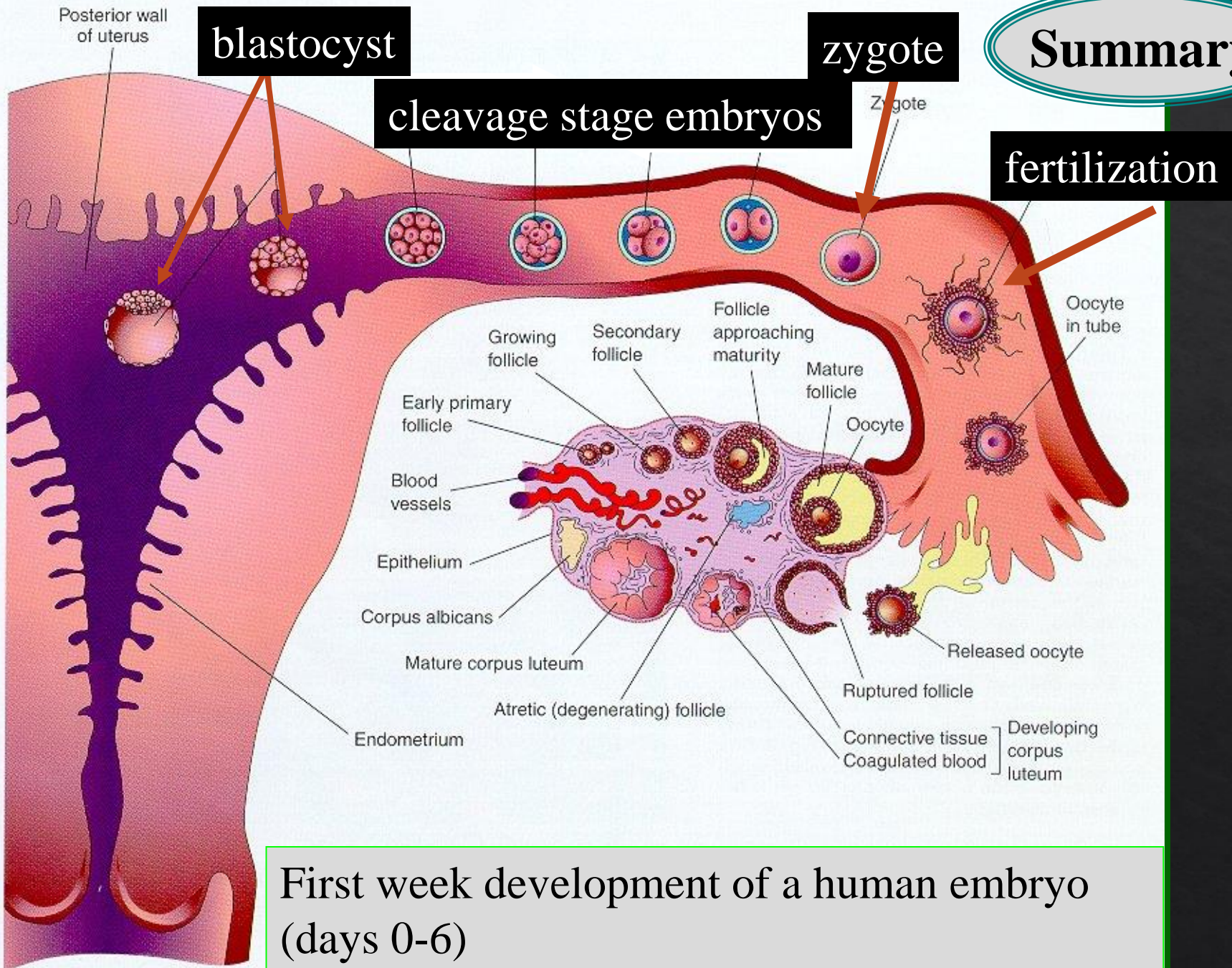
Chance of realisation	1-child family	2-child family	3-child family
Without IVF			
50%	41	38	35
75%	37	34	31
90%	32	27	23
With IVF			
50%	42	39	36
75%	39	35	33
90%	35	31	28

IVF WORKLOAD

In-vitro culture of the embryos for 2 to 5 days



Summary



First week development of a human embryo (days 0-6)

Learning objectives

- ◇ Overview of an IVF Center
- ◇ Approach to a infertile couple
- ◇ Methods used in infertility treatments
- ◇ Legal regulations, ethical concerns

Ankara University School of Medicine-IVF Laboratory



Ankara University School of Medicine-IVF Laboratory



Required units

Special air conditioning systems
Incubators
Microscopes
Micromanipulators
Laminar fluid cabins
Toxic gas filters
Freezing-thawing systems
Operating room

Staff

Gynecologists and obstetricians
Embryologists
Urologists
Nurses
Secretary and patient admission
Caregivers
Cleaning staff
Researchers-Interns

Approaching an infertile couple

- ◇ Evaluation of the male patient
 - ◇ Detailed history and examination
 - ◇ Endocrine examinations
 - ◇ Spermogram evaluation
 - ◇ **If necessary, evaluation of testicular sperm retrieval**
 - ◇ **Obstructive or non-obstructive azoospermia**

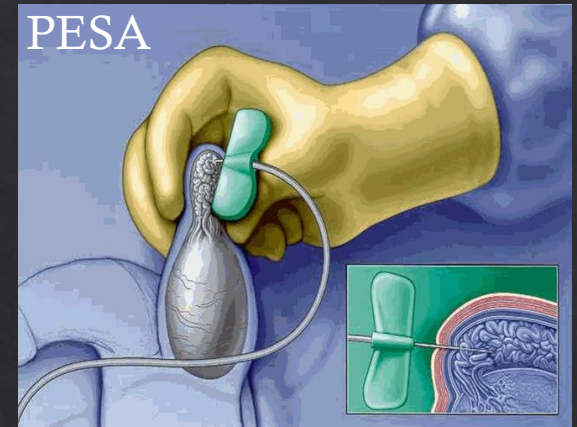


Table A1.1 Lower reference limits (5th centiles and their 95% confidence intervals) for semen characteristics

Parameter	Lower reference limit
Semen volume (ml)	1.5 (1.4–1.7)
Total sperm number (10^6 per ejaculate)	39 (33–46)
Sperm concentration (10^6 per ml)	15 (12–16)
Total motility (PR + NP, %)	40 (38–42)
Progressive motility (PR, %)	32 (31–34)
Vitality (live spermatozoa, %)	58 (55–63)
Sperm morphology (normal forms, %)	4 (3.0–4.0)
<i>Other consensus threshold values</i>	
pH	≥ 7.2
Peroxidase-positive leukocytes (10^6 per ml)	< 1.0
MAR test (motile spermatozoa with bound particles, %)	< 50
Immunobead test (motile spermatozoa with bound beads, %)	< 50
Seminal zinc (μmol /ejaculate)	≥ 2.4
Seminal fructose (μmol /ejaculate)	≥ 13
Seminal neutral glucosidase (mU/ejaculate)	≥ 20

Table A1.3 Nomenclature related to semen quality

aspermia	no semen (no or retrograde ejaculation)
asthenozoospermia	percentage of progressively motile (PR) spermatozoa below the lower reference limit
asthenoteratozoospermia	percentages of both progressively motile (PR) and morphologically normal spermatozoa below the lower reference limits
azoospermia	no spermatozoa in the ejaculate (given as the limit of quantification for the assessment method employed)
cryptozoospermia	spermatozoa absent from fresh preparations but observed in a centrifuged pellet
haemospermia (haemospermia)	presence of erythrocytes in the ejaculate
leukospermia (leukocytospermia, pyospermia)	presence of leukocytes in the ejaculate above the threshold value
necrozoospermia	low percentage of live, and high percentage of immotile, spermatozoa in the ejaculate
normozoospermia	total number (or concentration, depending on outcome reported)* of spermatozoa, and percentages of progressively motile (PR) and morphologically normal spermatozoa, equal to or above the lower reference limits
oligoasthenozoospermia	total number (or concentration, depending on outcome reported)* of spermatozoa, and percentage of progressively motile (PR) spermatozoa, below the lower reference limits
oligoasthenoteratozoospermia	total number (or concentration, depending on outcome reported)* of spermatozoa, and percentages of both progressively motile (PR) and morphologically normal spermatozoa, below the lower reference limits
oligoteratozoospermia	total number (or concentration, depending on outcome reported)* of spermatozoa, and percentage of morphologically normal spermatozoa, below the lower reference limits
oligozoospermia	total number (or concentration, depending on outcome reported)* of spermatozoa below the lower reference limit
teratozoospermia	percentage of morphologically normal spermatozoa below the lower reference limit

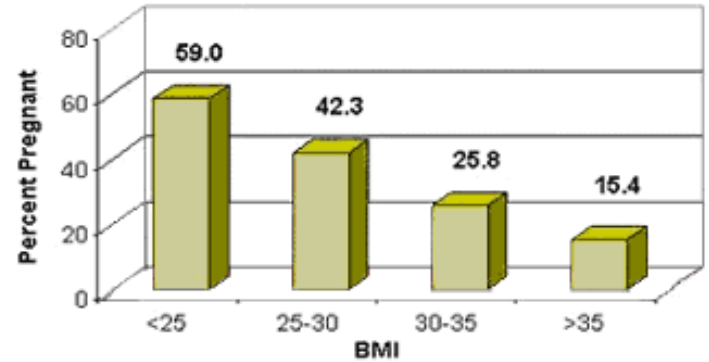
Approaching an infertile couple

- ◇ Evaluation of female patient
- ◇ Detailed history and physical examination
 - ◇ **Age**, duration of the marriage, previous pregnancies, abortions, **body mass index** (BMI), smoking history, previous (chronic) diseases and surgery, any medication
- ◇ Endocrine evaluation
 - ◇ Evaluation of the ovarian reserve
 - ◇ Estradiol, progesterone, prolactin, FSH, LH, **Anti-Mullerian Hormone** (AMH, norm. 1-2 mg/mL), **Antral Follicle Count** (AFC, norm. 11-30 follicles)
- ◇ Evaluation of the uterine cavity
 - ◇ 3D transvaginal ultrasonography
 - ◇ Histerosalphyngography

Body Mass Index - (Kg/m²)

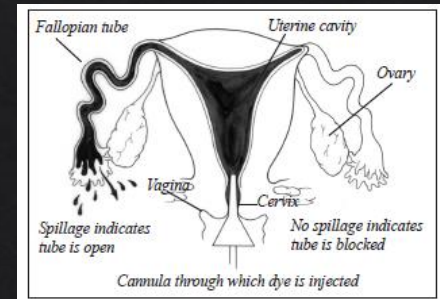
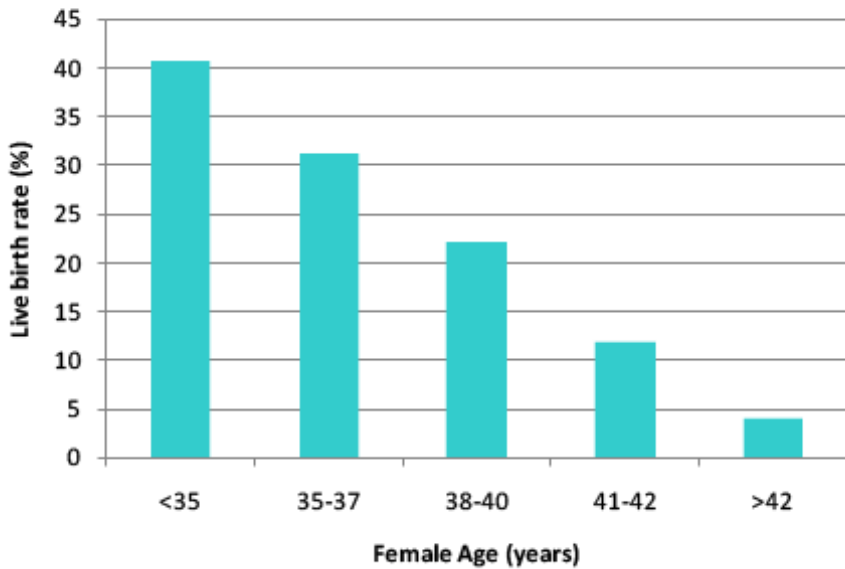


IVF Rates Per Retrieval & BMI - Age Under 40 August 2000 - March 2002

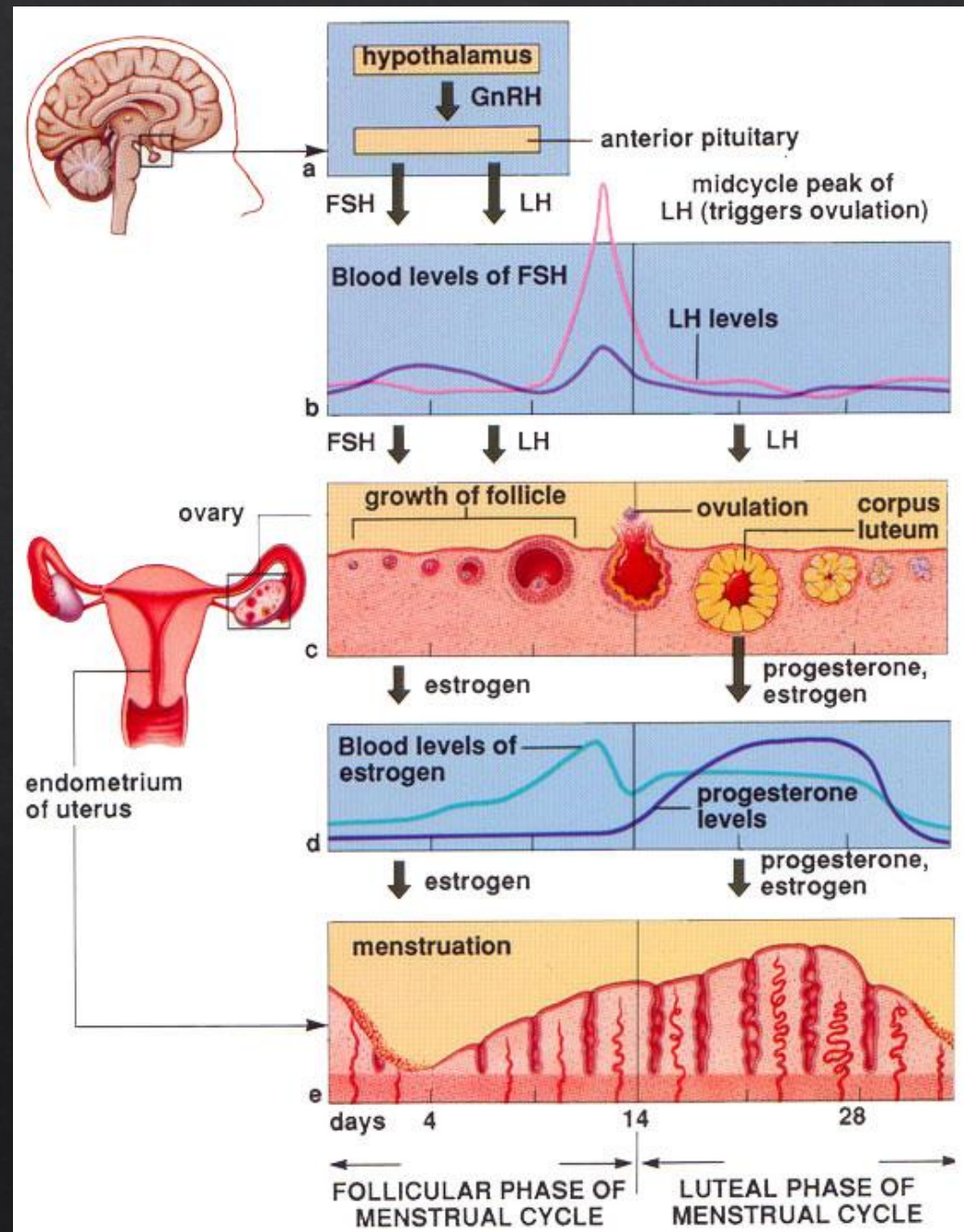


(Data is for all women under 40 having IVF at AFC Chicago between August 2000 and March 2002)

2012 live birth rate after IVF



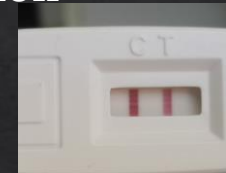
1. Menstrual bleeding
2. Development and selection of follicles in the ovary: FSH and LH dependent, **estrogen** secreted during development
3. When the amount of estrogen reaches the highest level, LH release triggered from the pituitary and **LH peak** occurs.
4. The excessive increase of LH causes the follicle in the ovary to ovulate, oocyte expulsion and the termination of **Meiosis-I: Ovulation. Product: Mature (M-II) oocyte**
5. The left over granulosa cells are luteinized and the **corpus luteum** is formed. The corpus luteum secretes **progesterone**.
6. Increased estrogen at the beginning of the cycle, the and progesterone secreted from CL develop the endometrium and the **implantation window** opens.





The embryo is embedded in the endometrium: implantation

Cells that will form the placenta secrete hCG

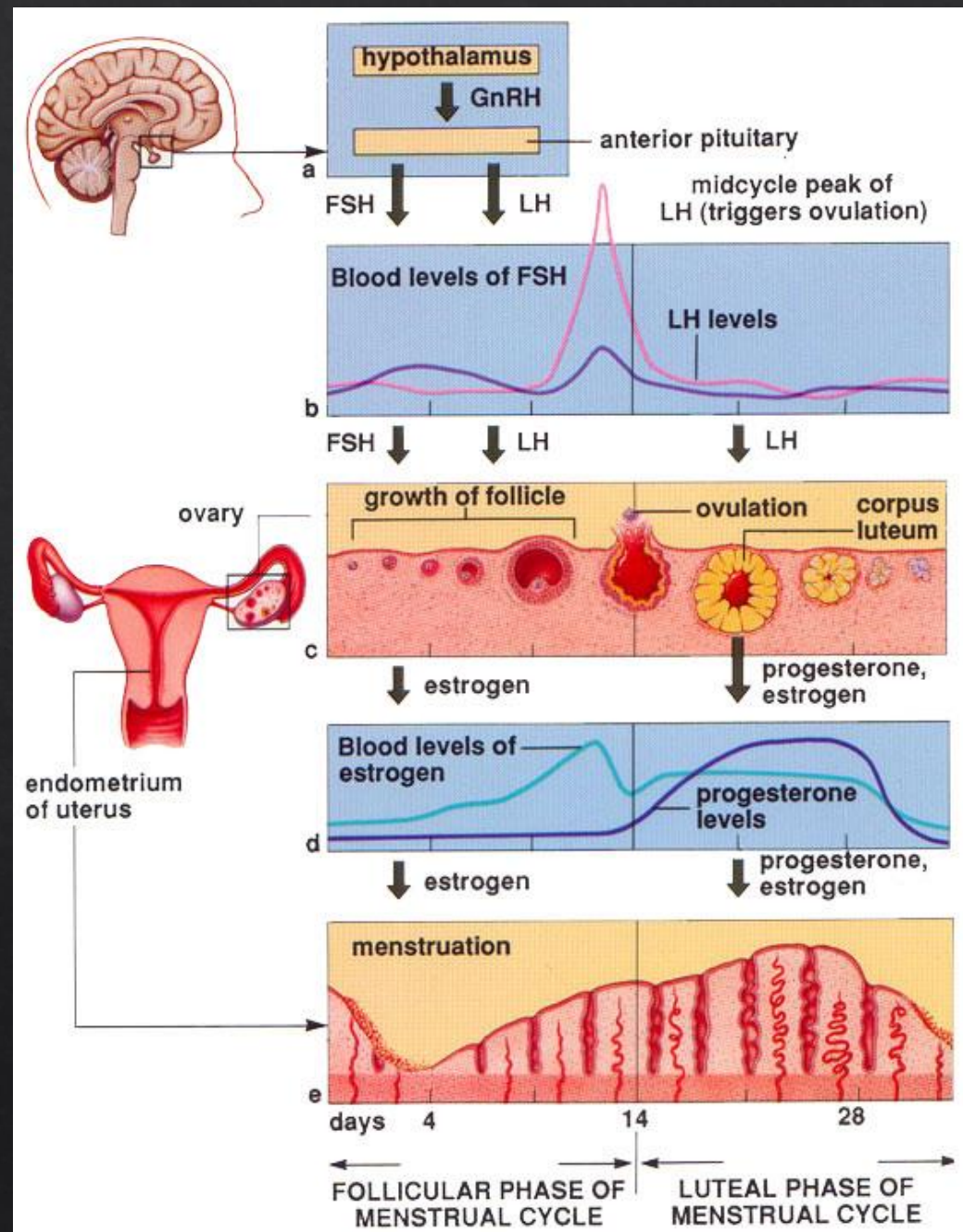


hCG provides progesterone release by stimulating the corpus luteum (corpus luteum of the pregnancy)

Endometrium is supported, pregnancy continues



1. Menstrual bleeding
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7. The life span of the corpus luteum is about **14 days**, it degenerates when not supported.
8. When the progesterone source disappears, the functional layer of the endometrium is separated from its basal layer.
9. **Menstrual bleeding**



CONTROLLED OVARIAN HYPERSTIMULATION

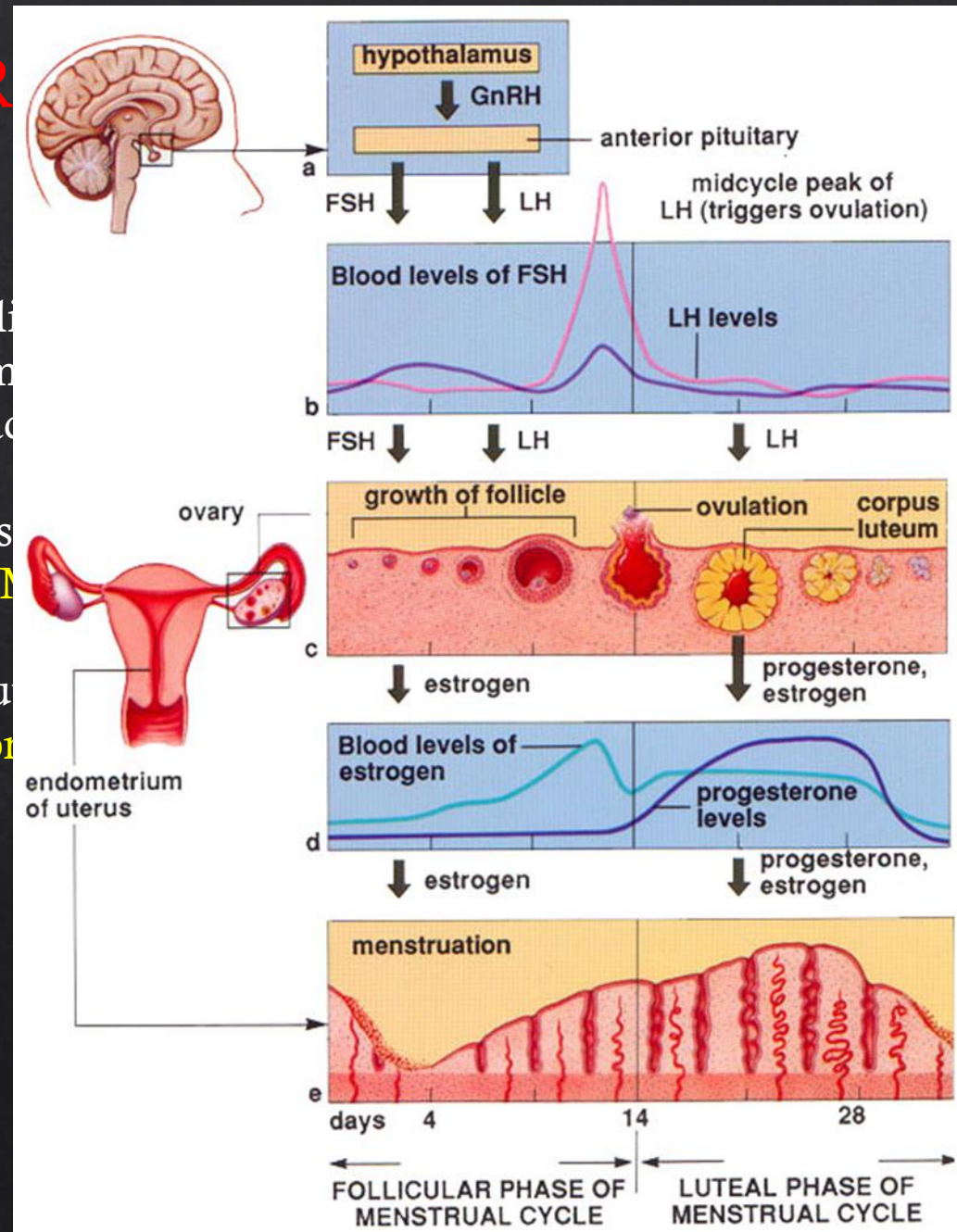
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CONTROLLED OVARIAN HYPERSTIMULATION

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- 6.
- 7.
- 8.
- 9.

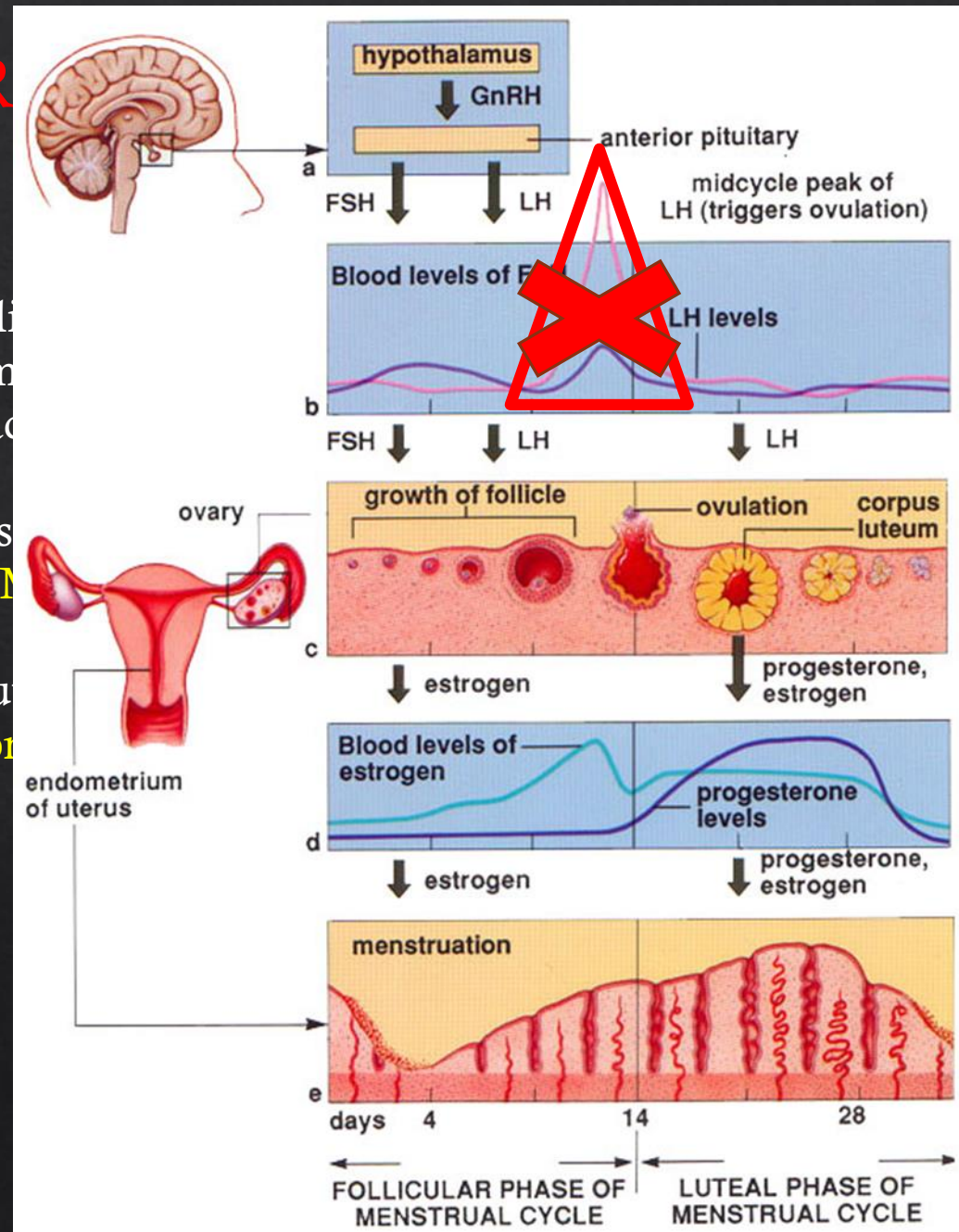
CONTROLLED OVAR

- 1.
2. Development and selection of follicle
3. **estrogen** secreted during development
4. When the amount of estrogen reaches a certain level, it stimulates the pituitary and **LH peak** occurs.
5. The excessive increase of LH causes ovulation and the termination of **ovulation**
6. **oocyte**
7. The left over granulosa cells are luteinized and the **corpus luteum** secretes **progesterone**
- 8.
- 9.



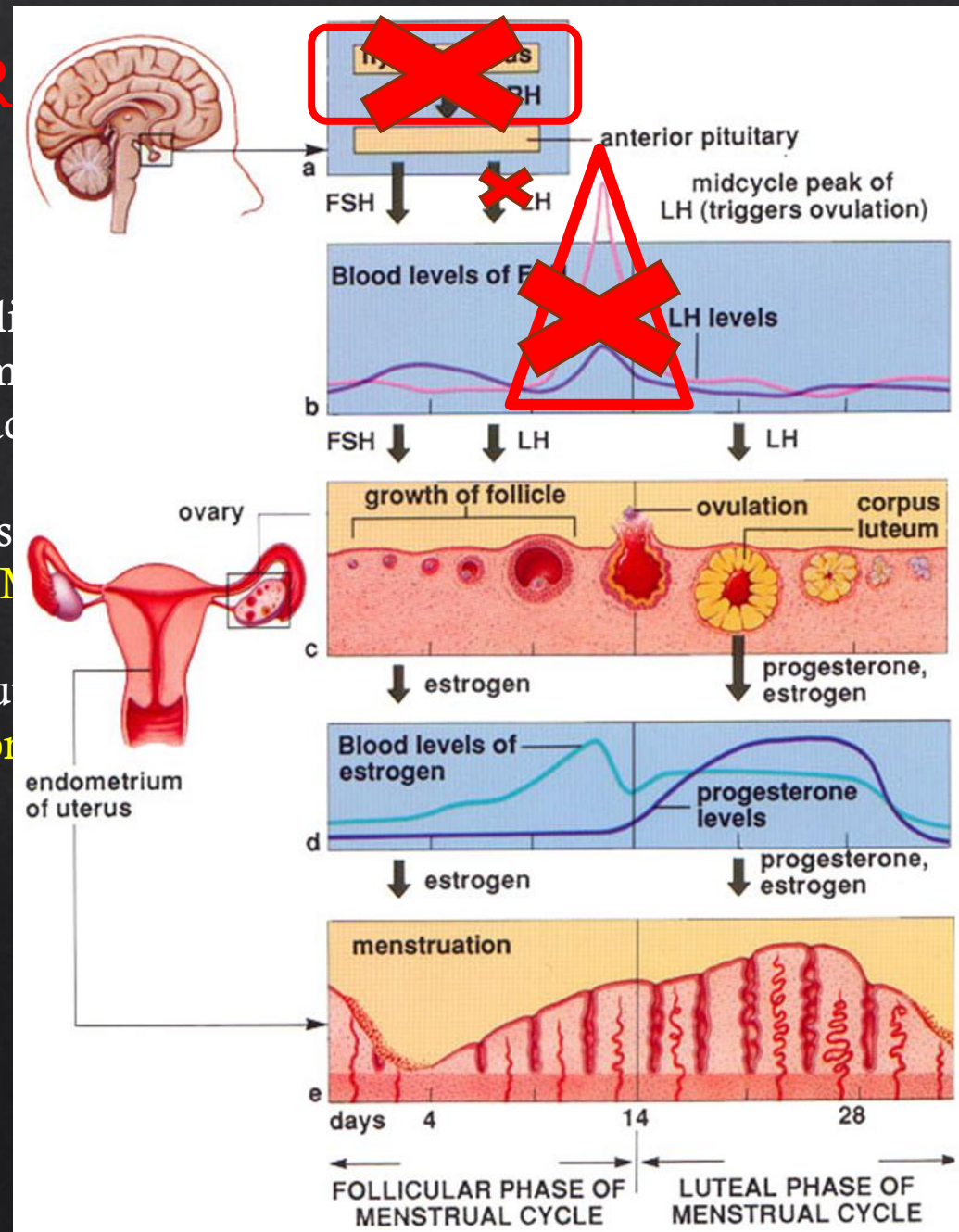
CONTROLLED OVAR

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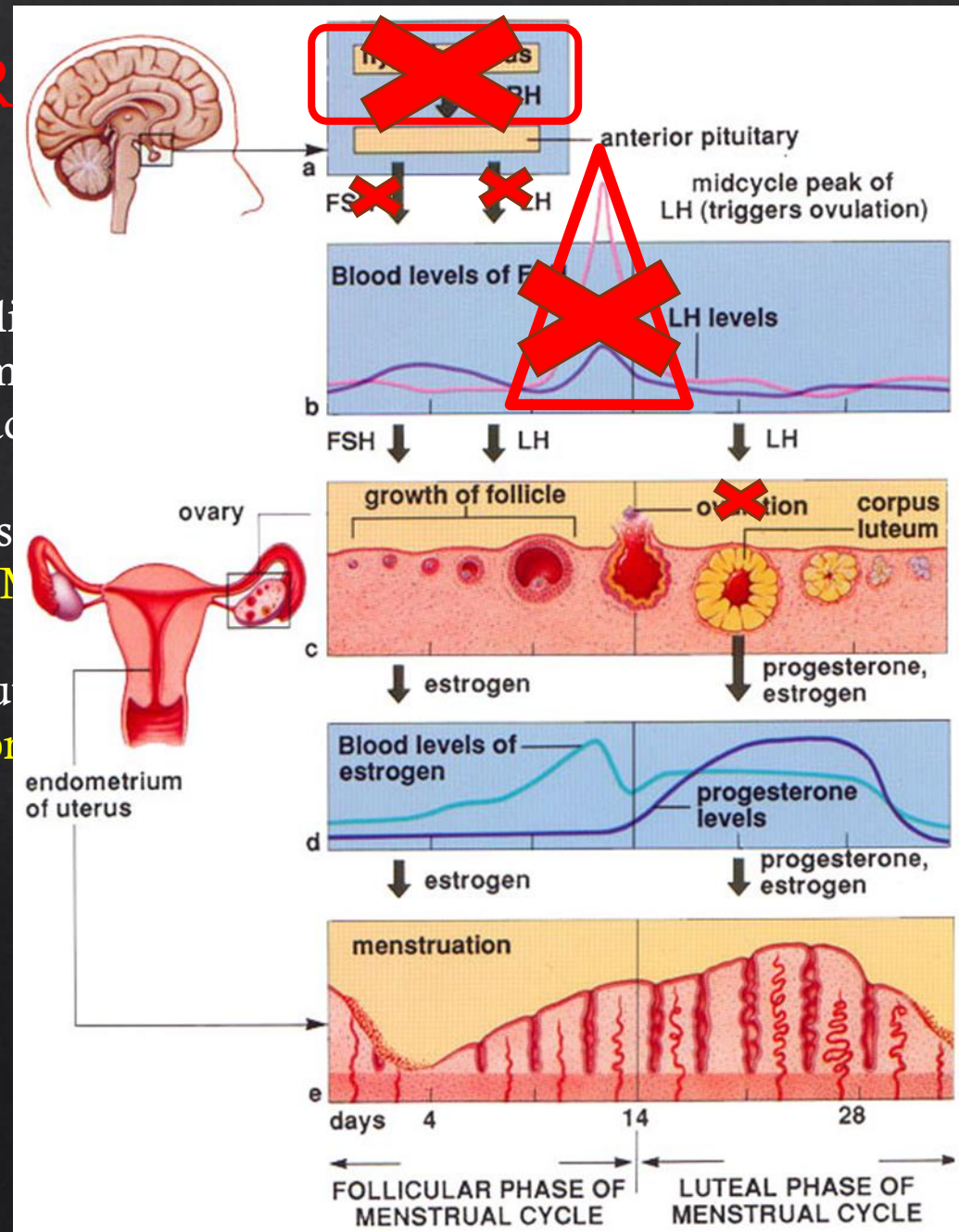
CONTROLLED OVAR

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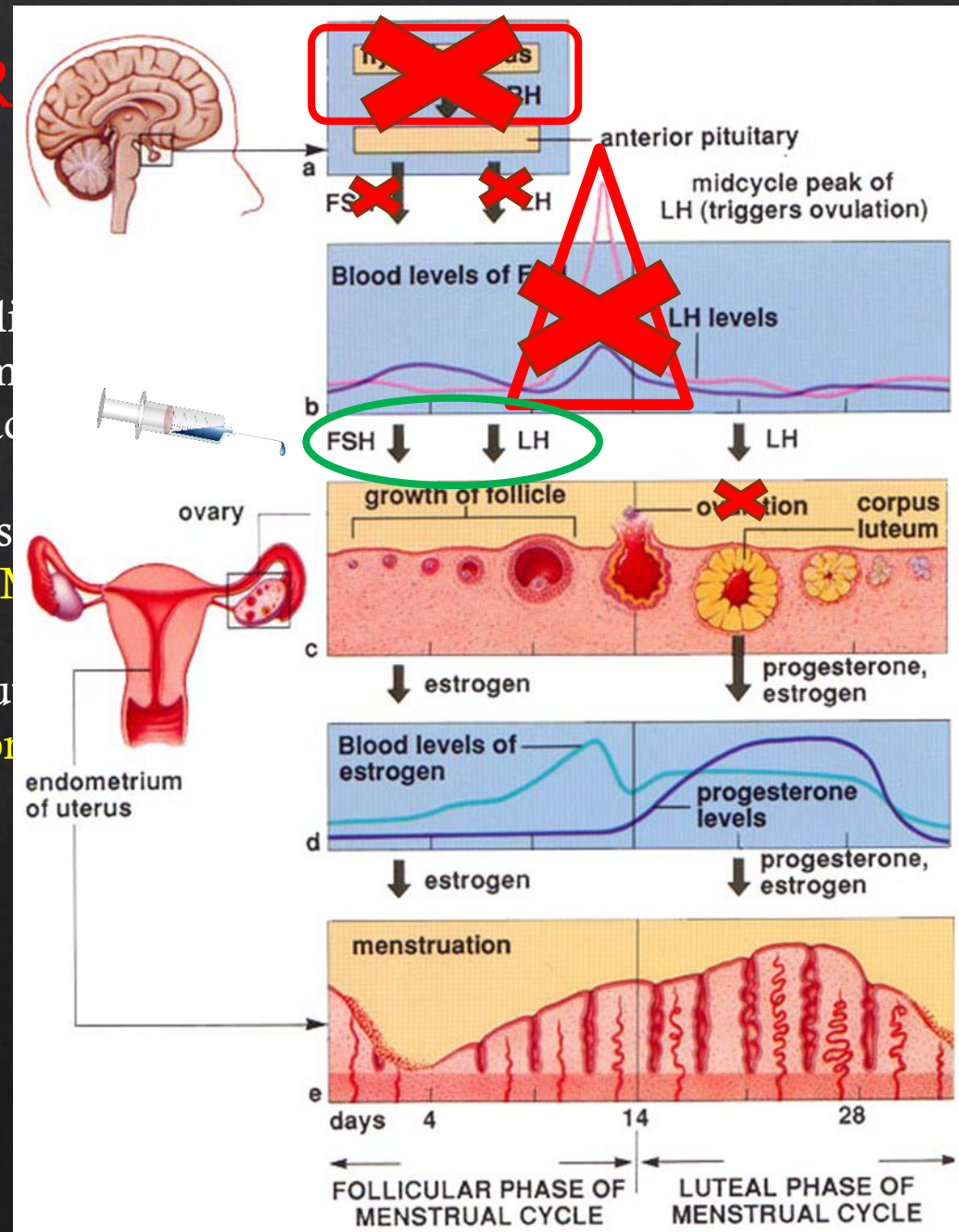
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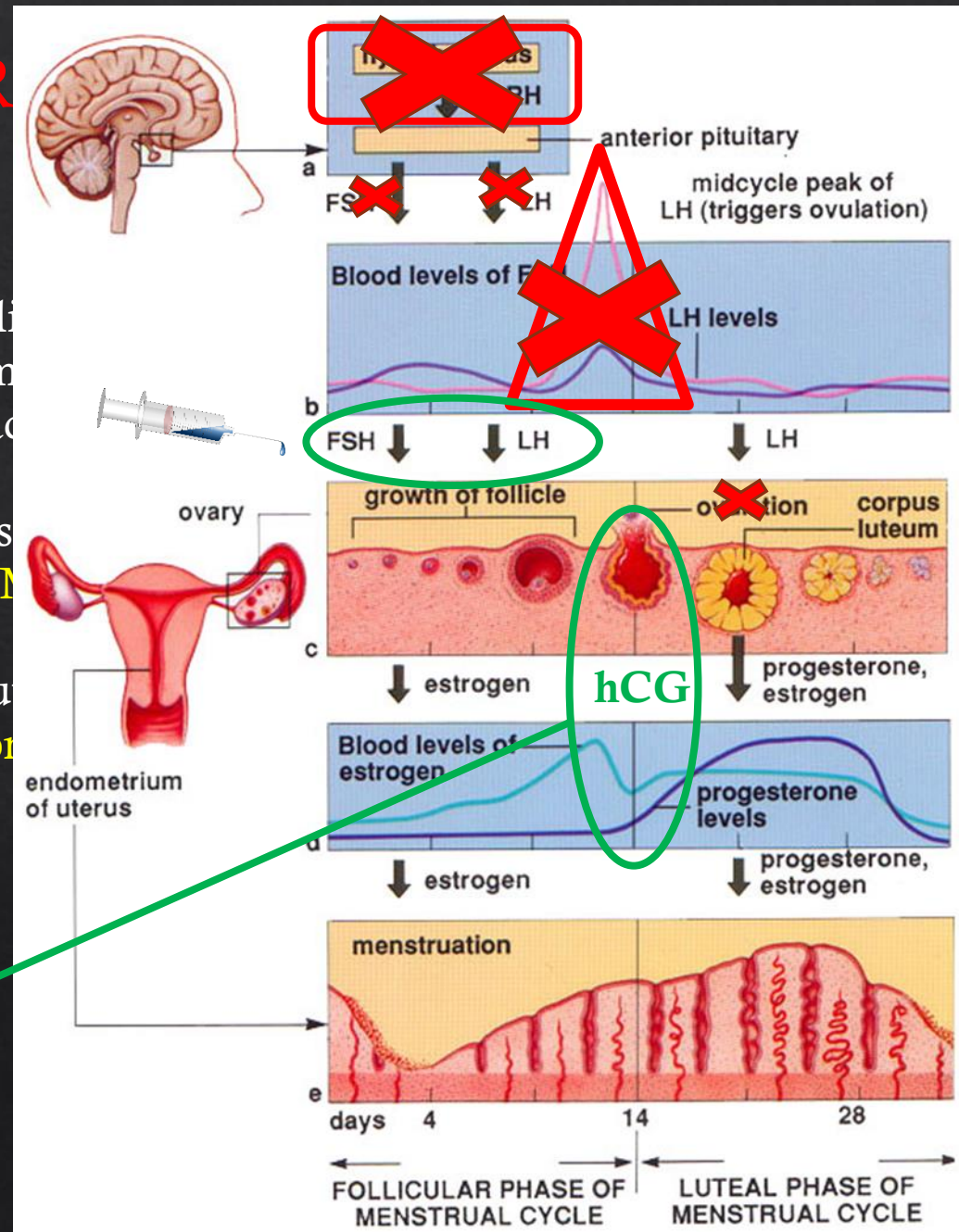
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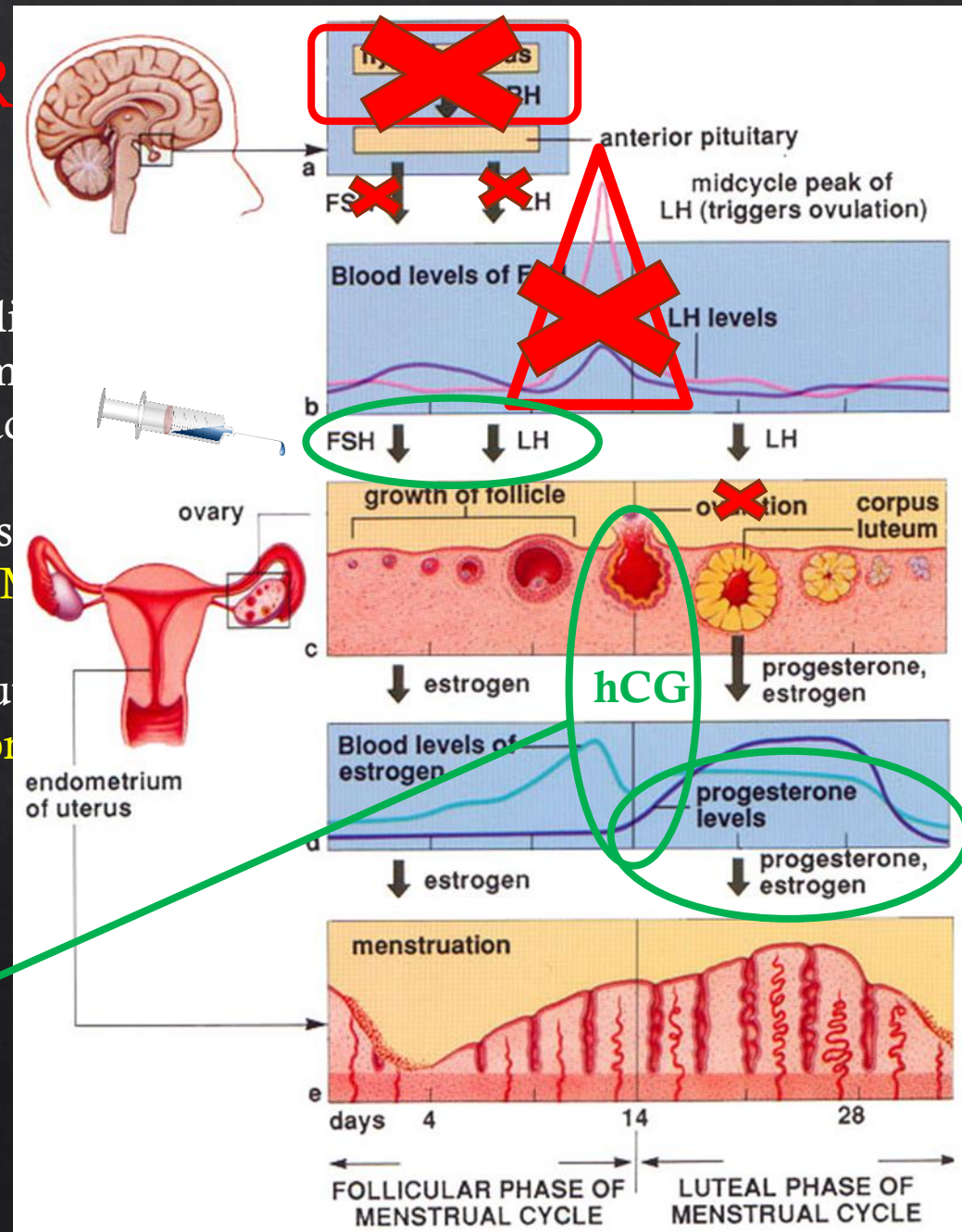
CONTROLLED OVARIAN

- 1.
2. Development and selection of follicle
3. When the amount of estrogen reaches a certain level, the anterior pituitary secretes **LH** and **FSH**.
4. The excessive increase of LH causes ovulation and the termination of the follicle.
5. The left over granulosa cells are luteinized and the corpus luteum secretes **progesterone** and **estrogen**.
- 6.
- 7.
- 8.
- 9.



CONTROLLED OVARIAN

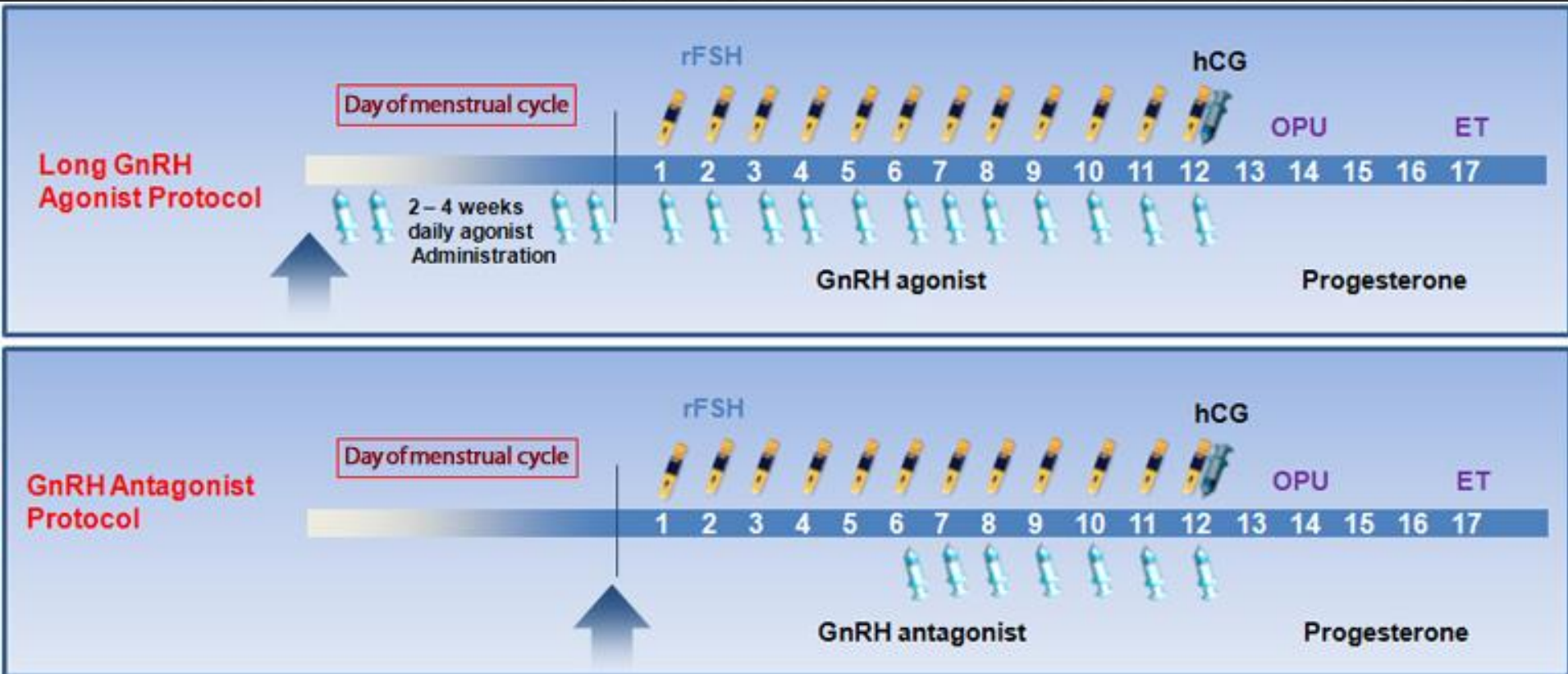
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Need to collect oocytes in 36 h

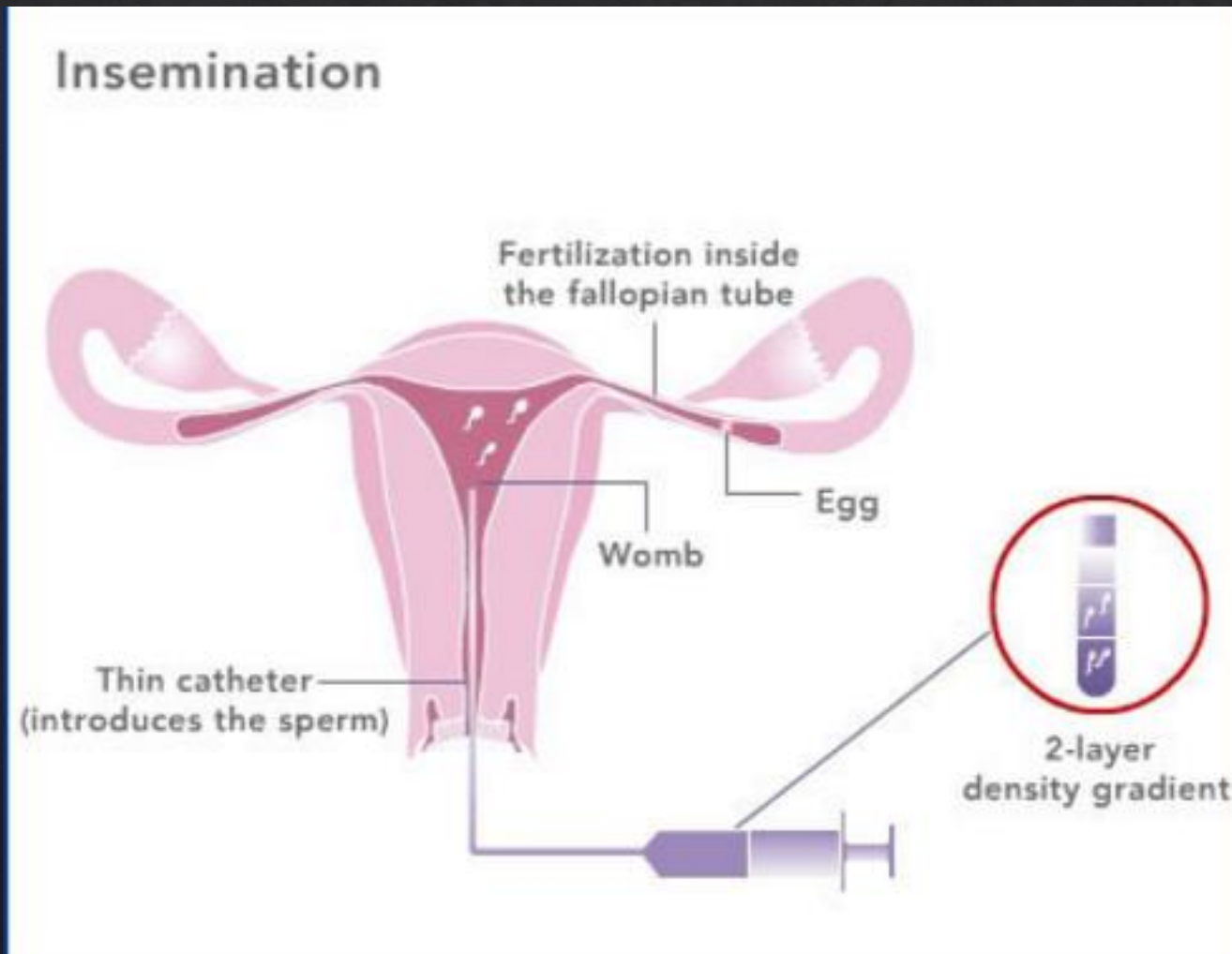
LUTEAL PHASE SUPPORT

COH PROTOCOLS



Treatment options

intrauterine insemination (IUI)



Treatment options *in-vitro fertilisation / ICSI*



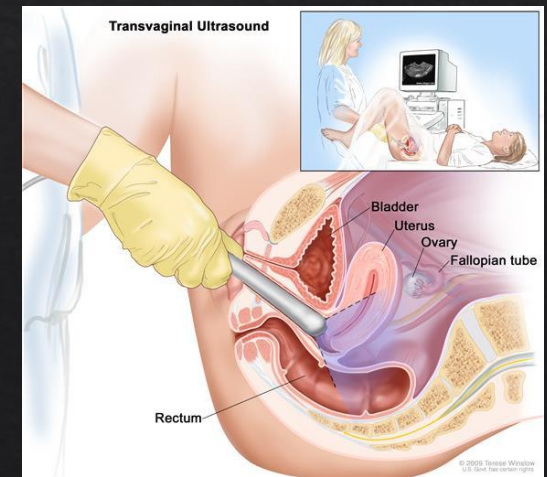
D2-3 of the menstrual cycle



Hormonal analyses



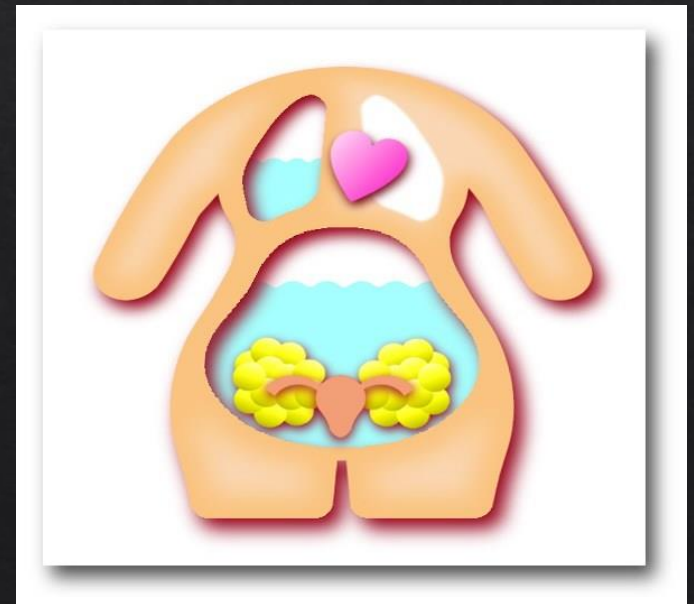
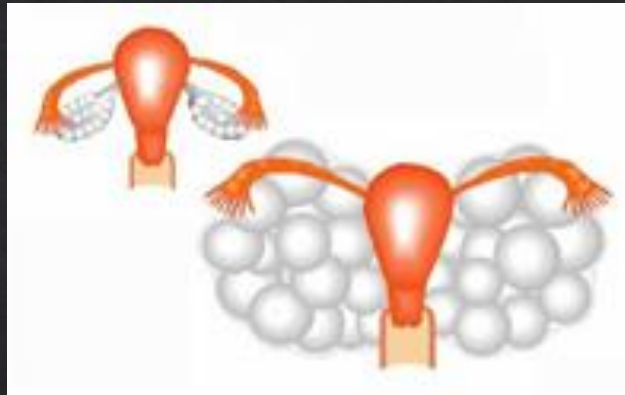
Transvaginal USG



When the
dominant
follicle
reaches
18-19 mm
hCG

Folliculometry

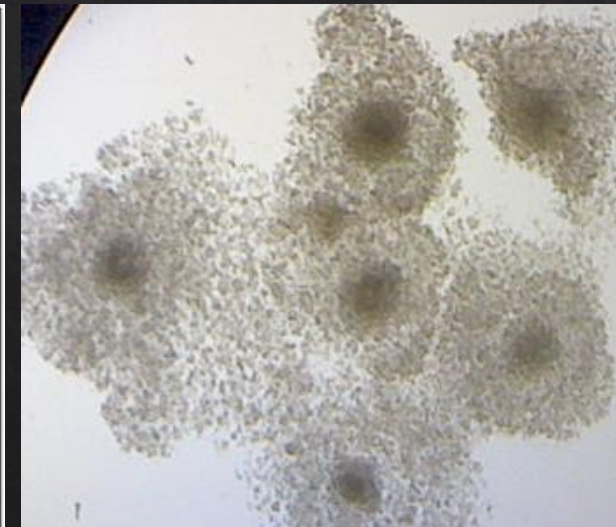
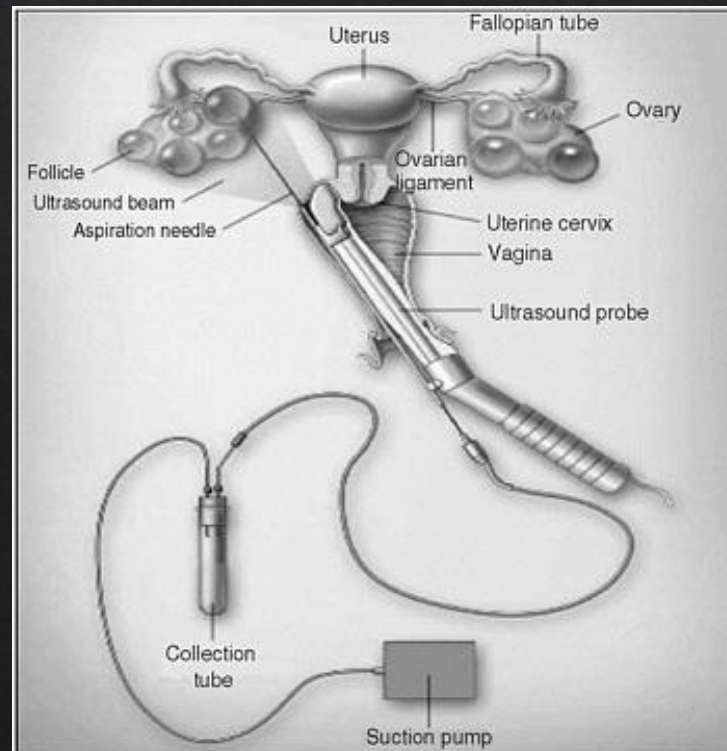
Ovarian hyperstimulation syndrome (OHSS)



Oocyte retrieval

oocyte pick-up (OPU)

- ❖ Oocytes that mature 35-36 hours after ovulation induction (trigger) are collected with appropriate needles by transvaginal ultrasound.



Cumulus-oocyte complex

Follicle aspiration

This is the first stage of the IVF process



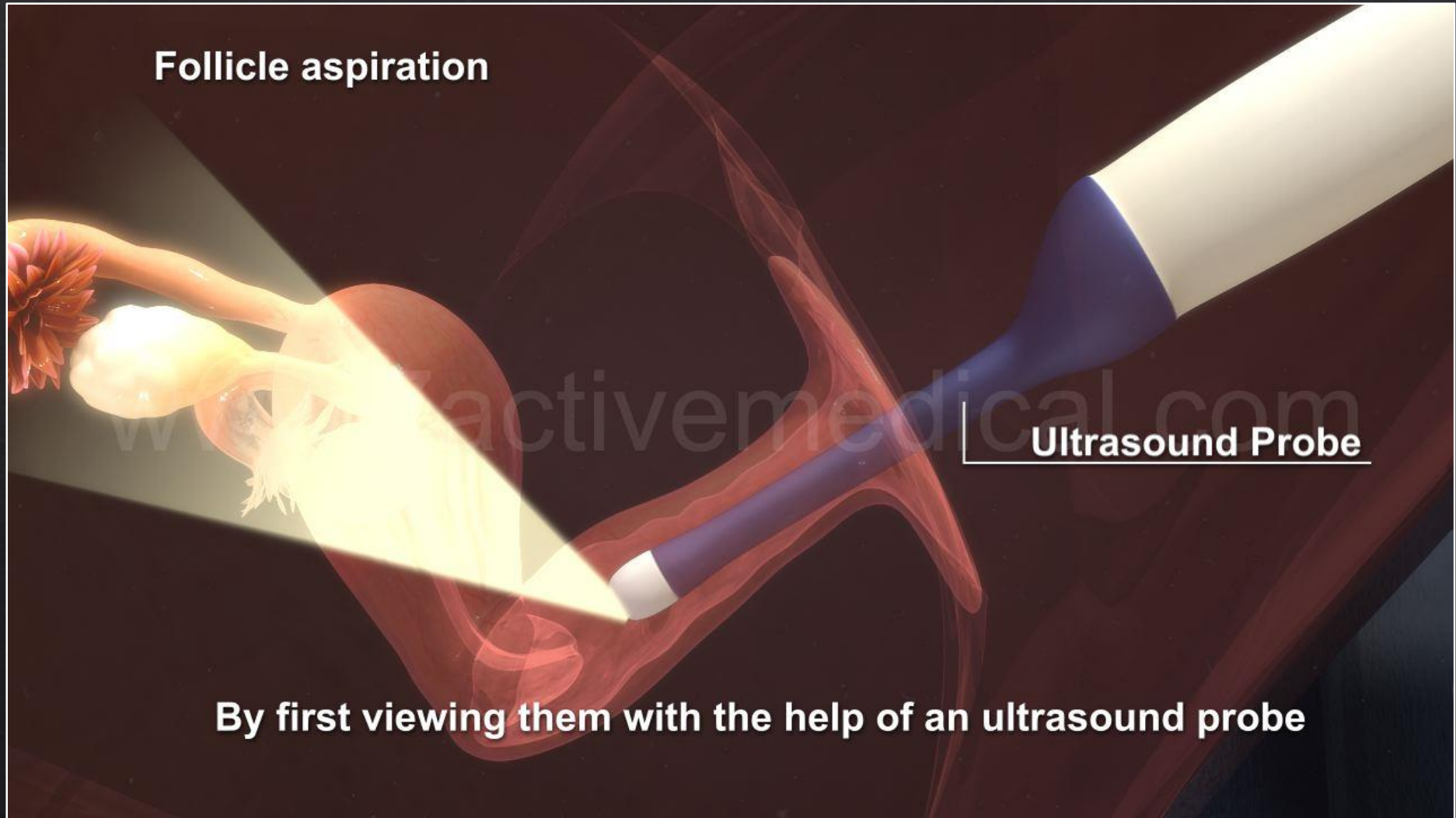
www.7activemedical.com

Follicle aspiration



In which the eggs are harvested from the ovaries

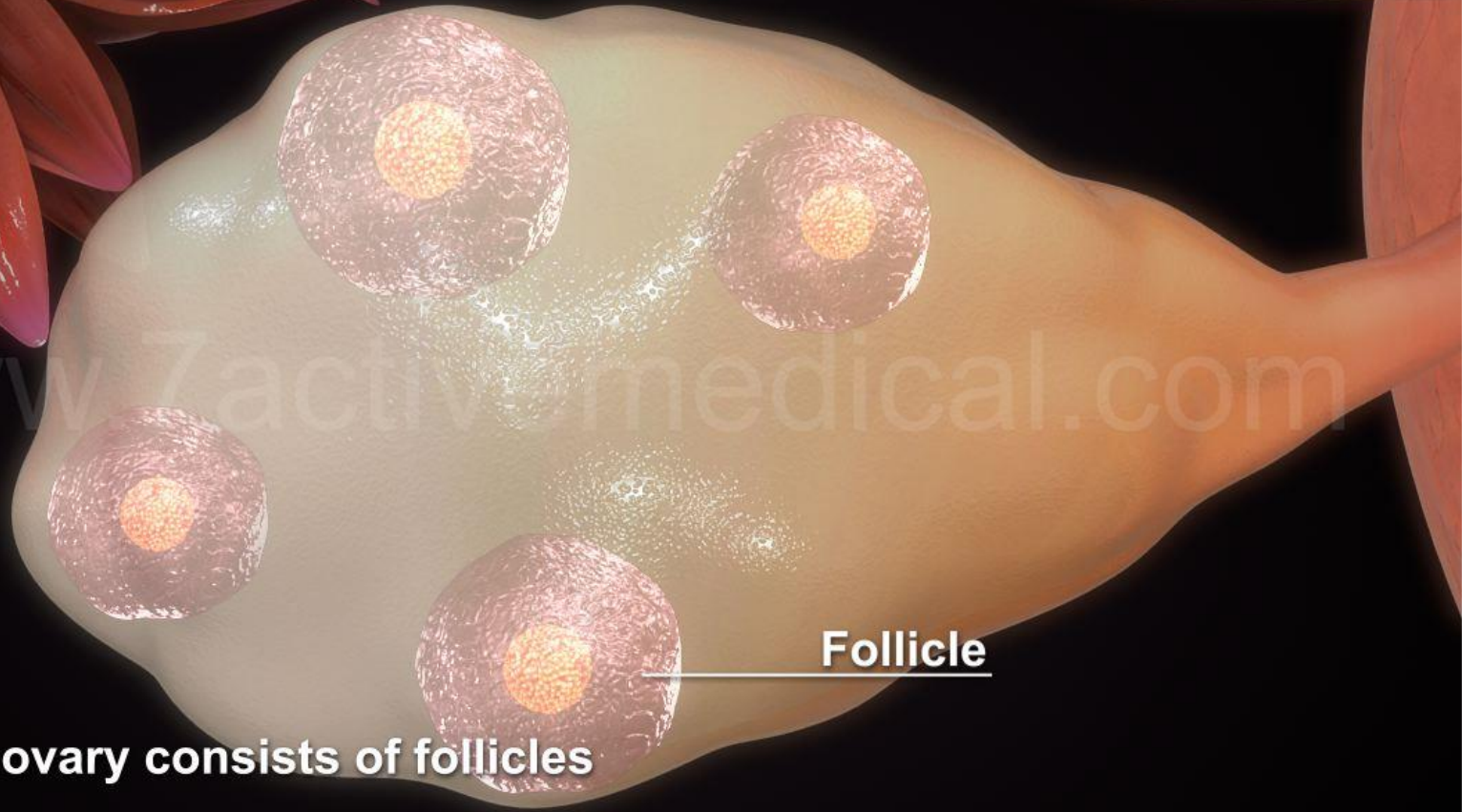
Follicle aspiration



Ultrasound Probe

By first viewing them with the help of an ultrasound probe

Follicle aspiration



Follicle

Each ovary consists of follicles

Follicle aspiration



Egg

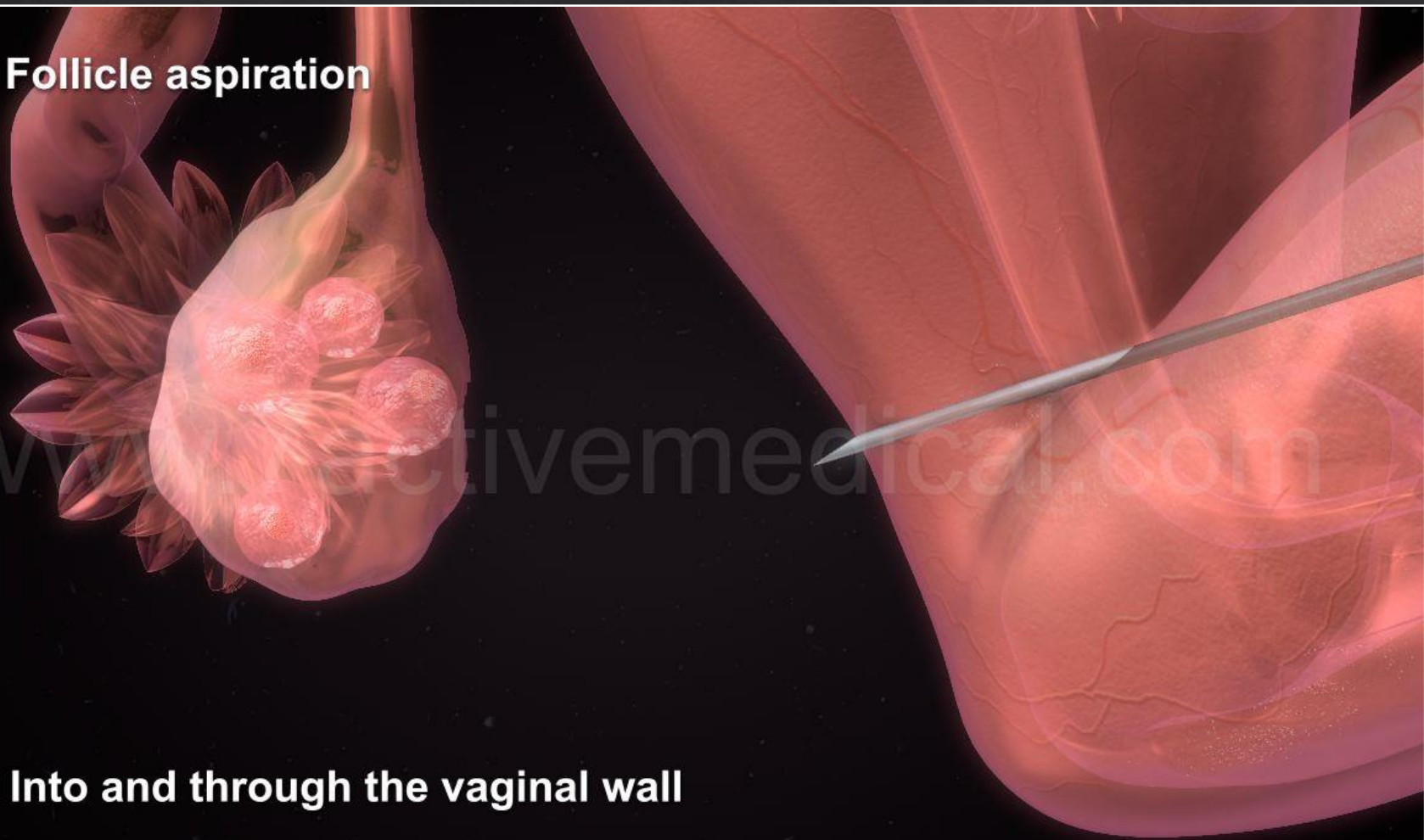
Which are fluid filled sacs containing the eggs needing to be collected.

Follicle aspiration



This is done through the insertion of a long, thin needle

Follicle aspiration

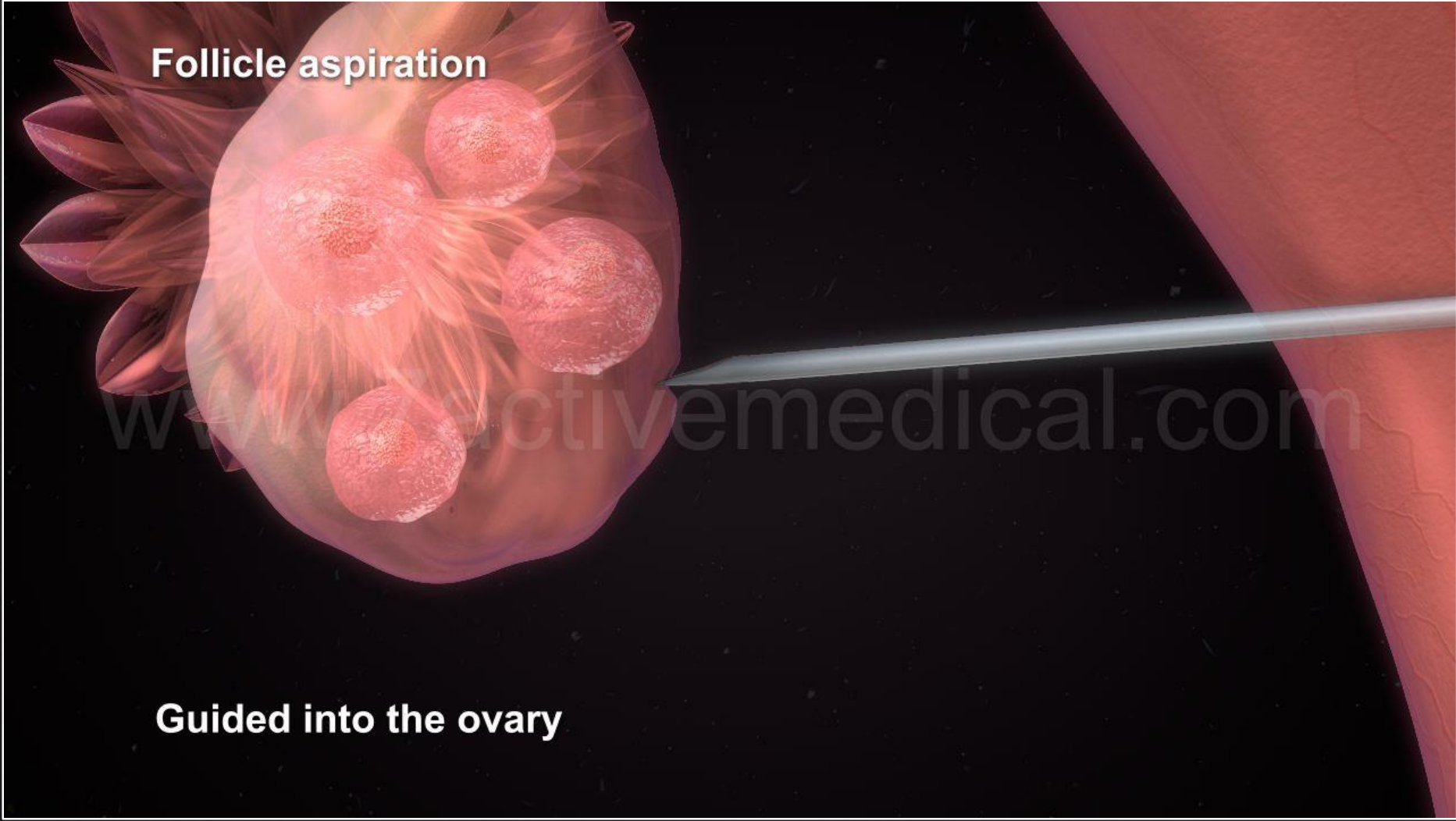


Into and through the vaginal wall

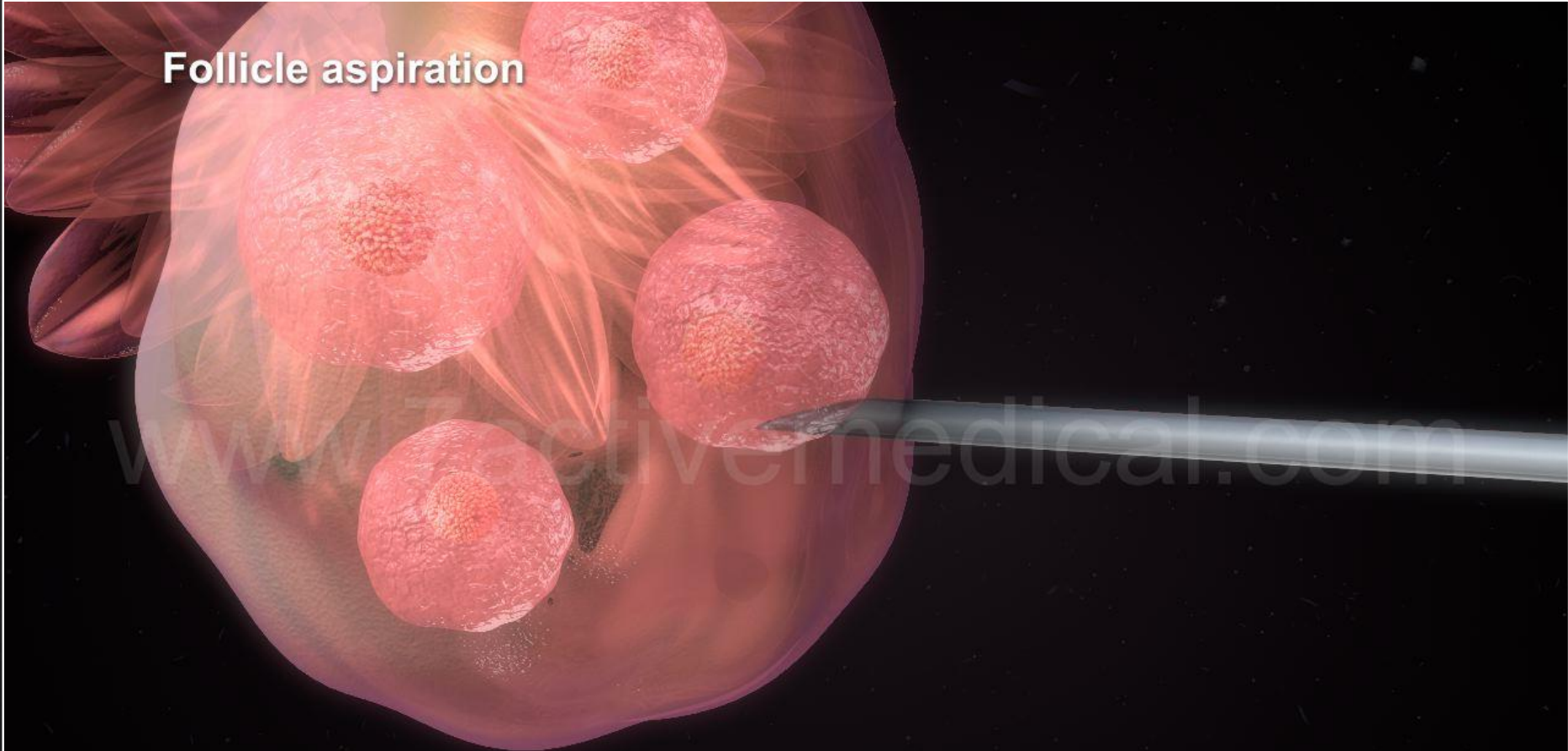
Follicle aspiration

Guided into the ovary

www.reactivemedical.com

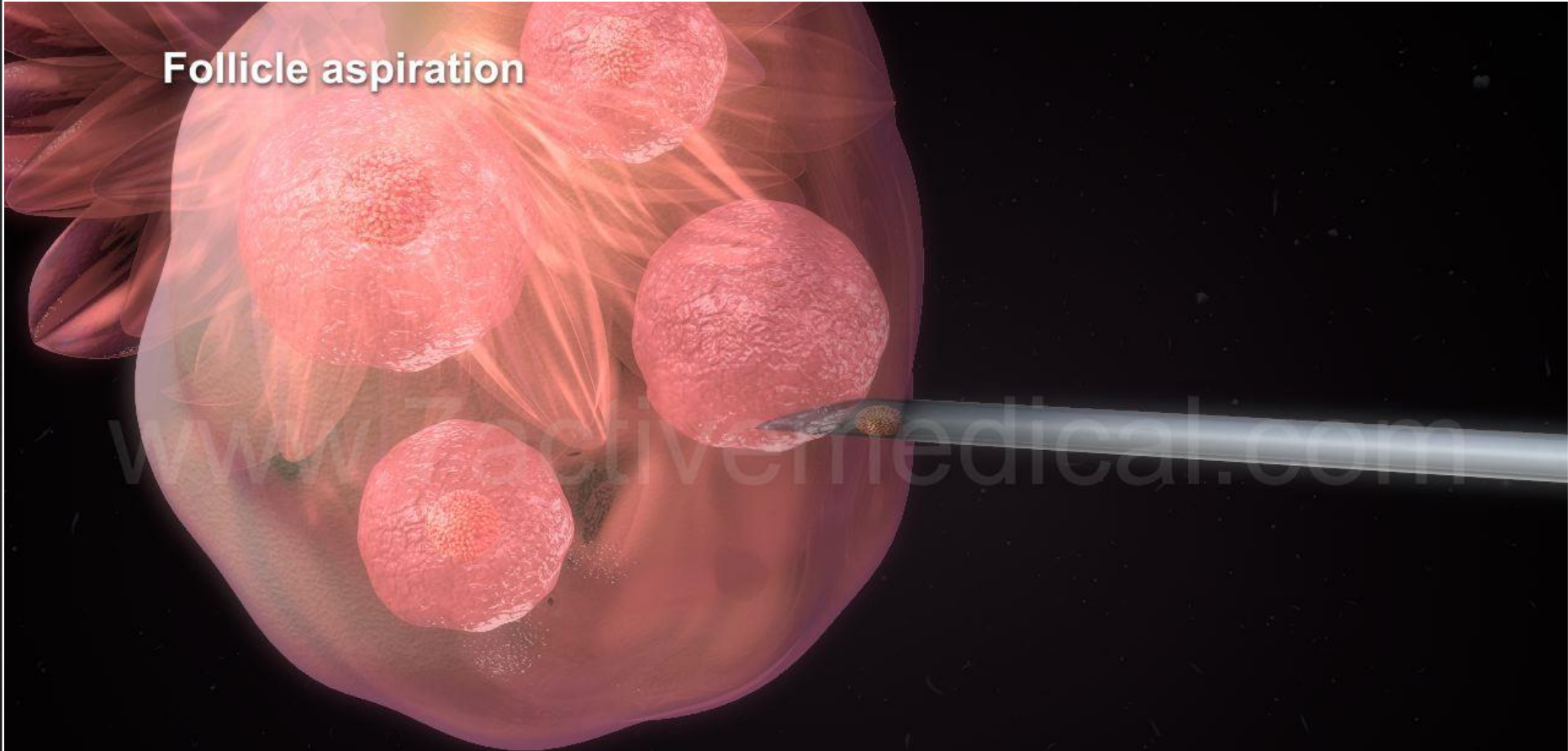


Follicle aspiration



A suction device is then connected to the needle

Follicle aspiration



Allowing the collection of the desired eggs, from inside the follicles

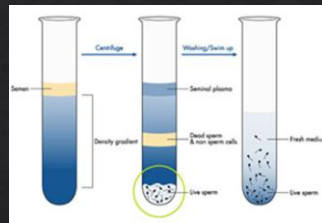


Sperm

Oocyte

Embryology Laboratory

Sperm washing and preparation

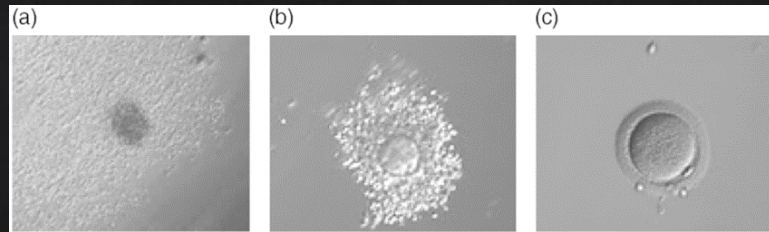


ICSI/
Classical IVF

Embryo culture

Embryo transfer

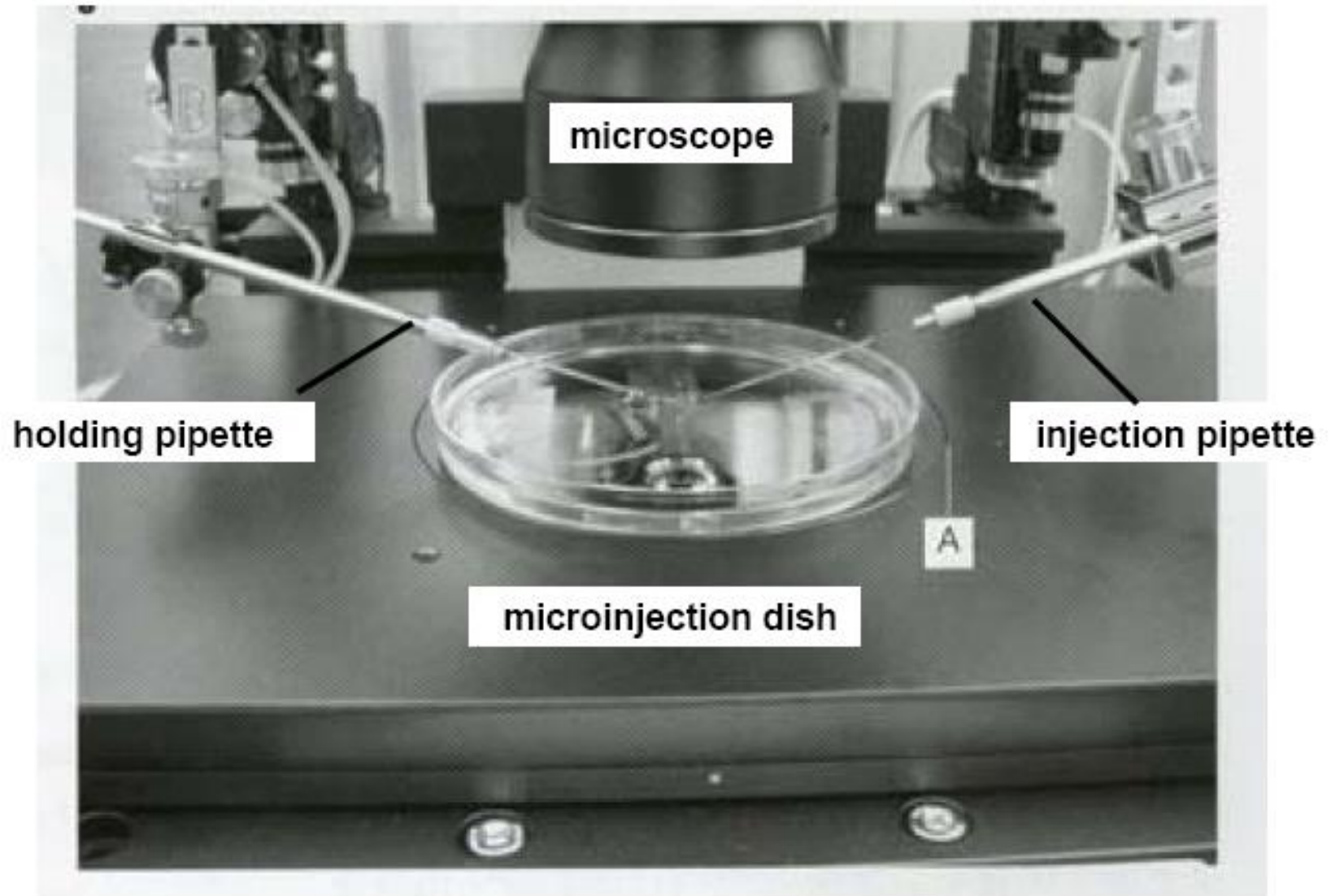
Oocyte denudation







micromanipulators for microinjection



ICSI





- **Extrusion of second polar body (completion of meiosis)**
- **Pronucleus formation**
- **Singamy**
- **Beginning of mitosis**

16-18th hour



Sperm
entering



Cortical
reaction

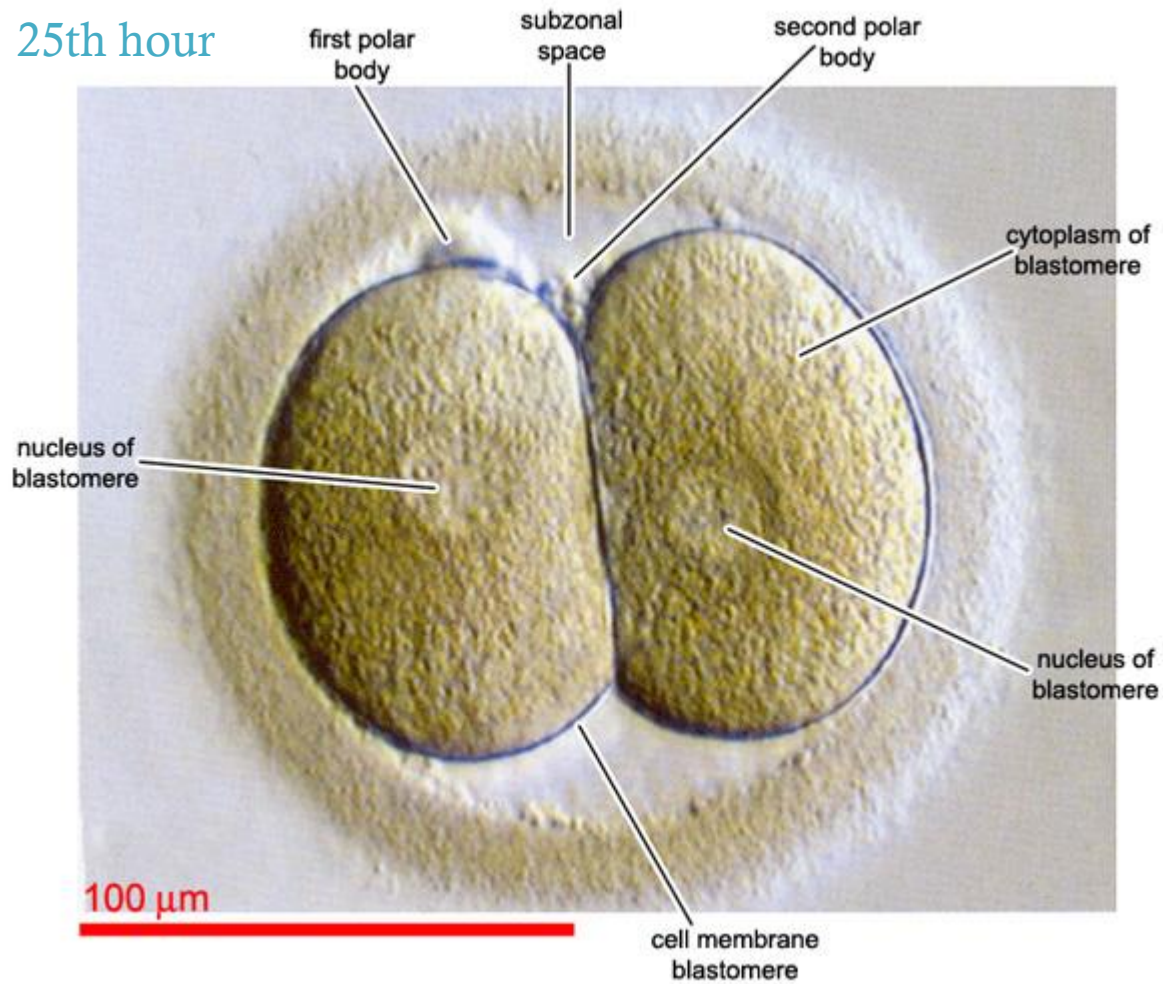


Extrusion of
second polar body
(completion of
meiosis)

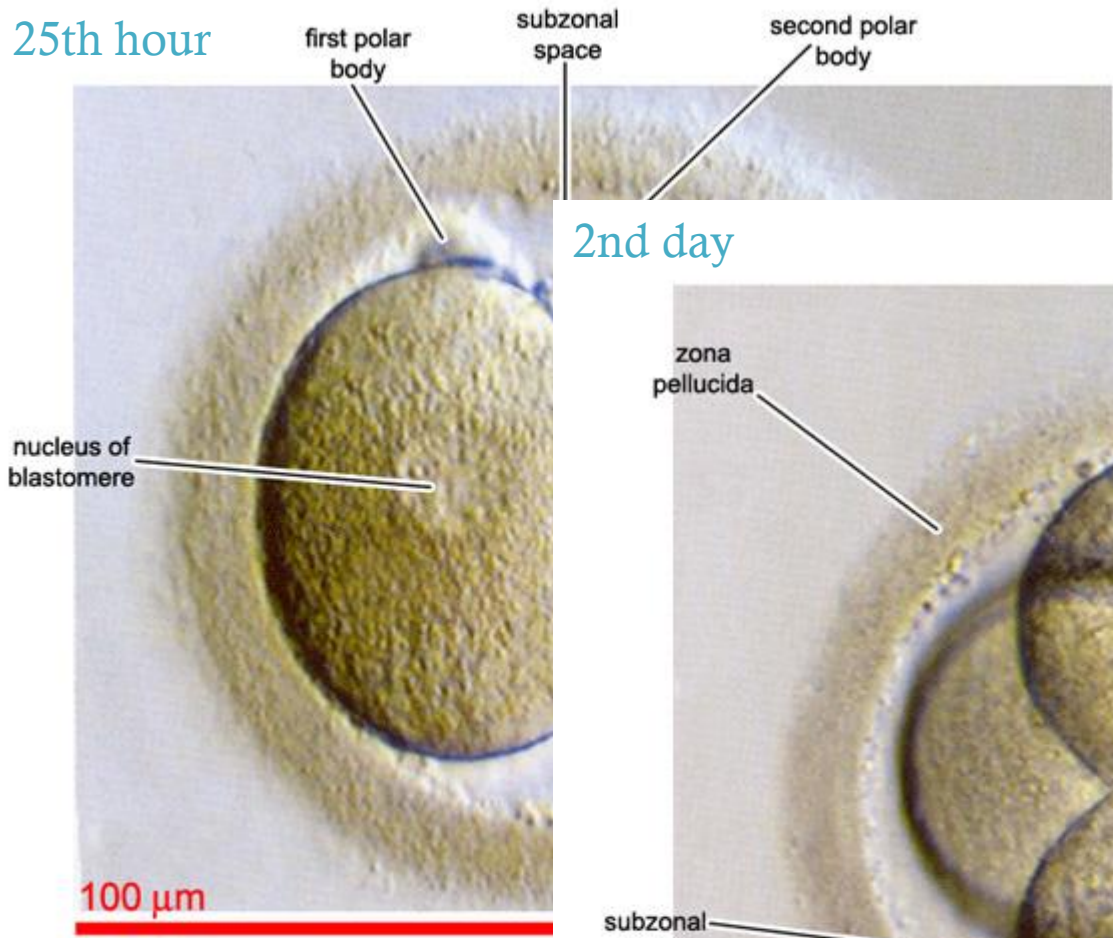


2 PN formation
(zygote)

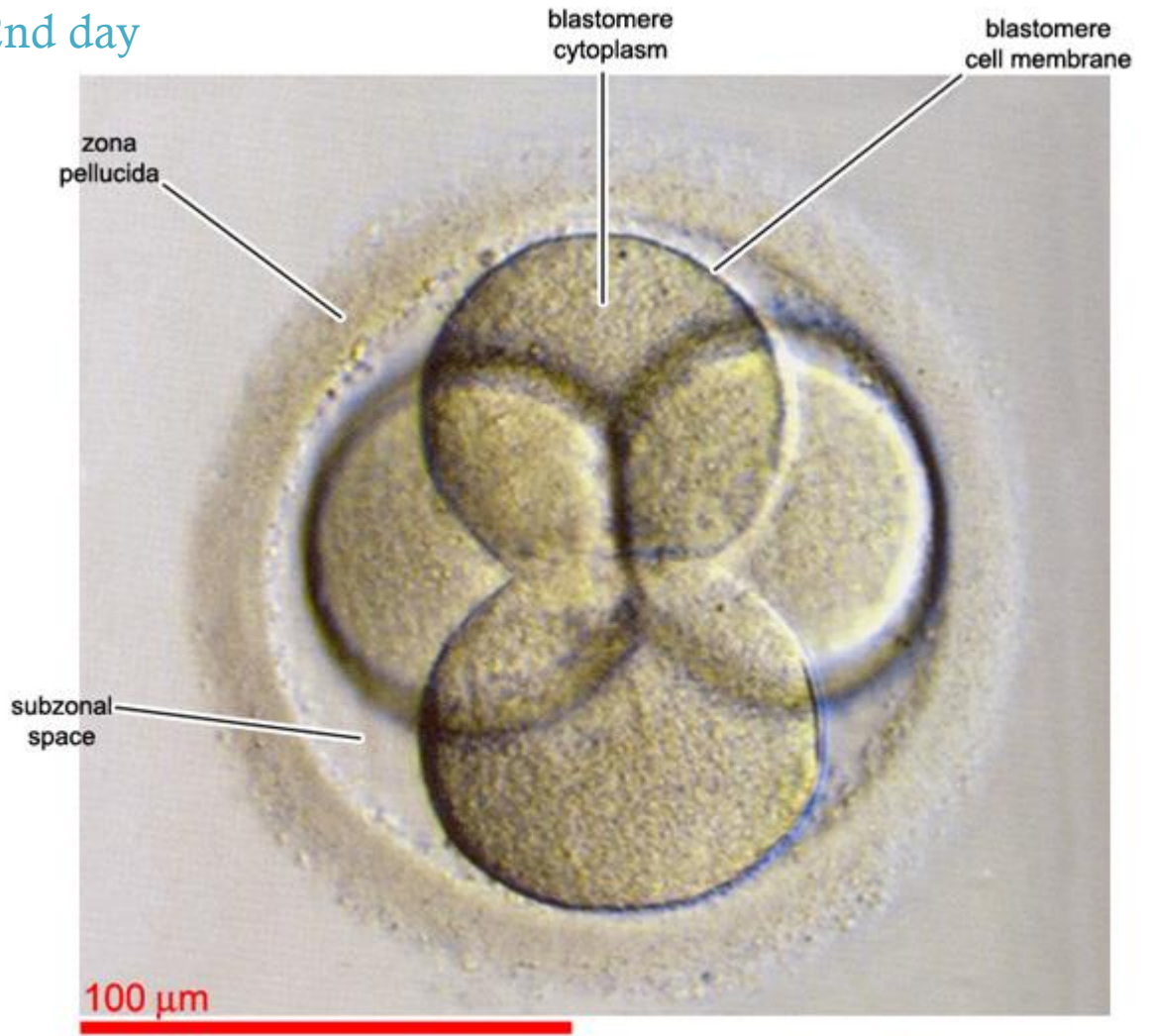
25th hour



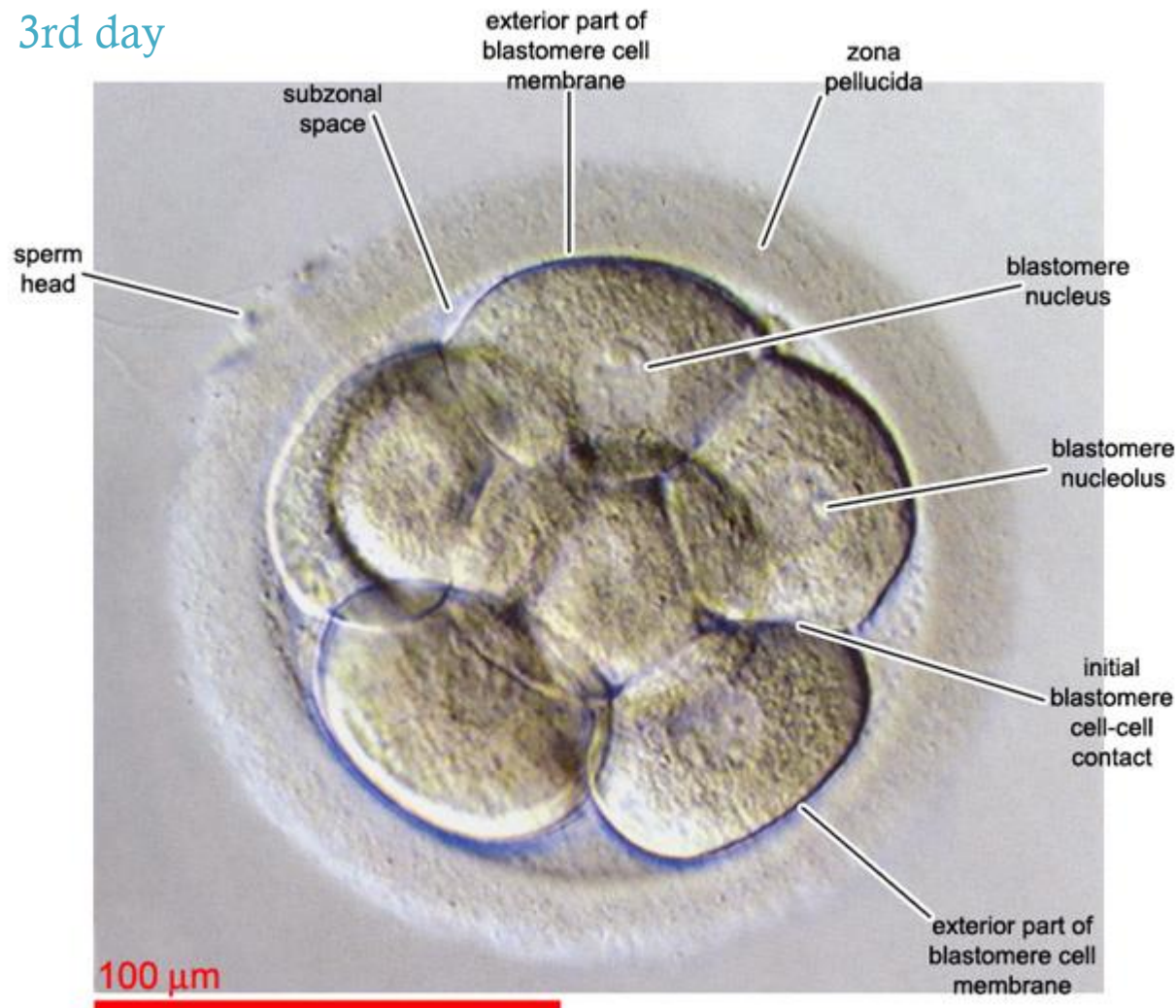
25th hour



2nd day



3rd day

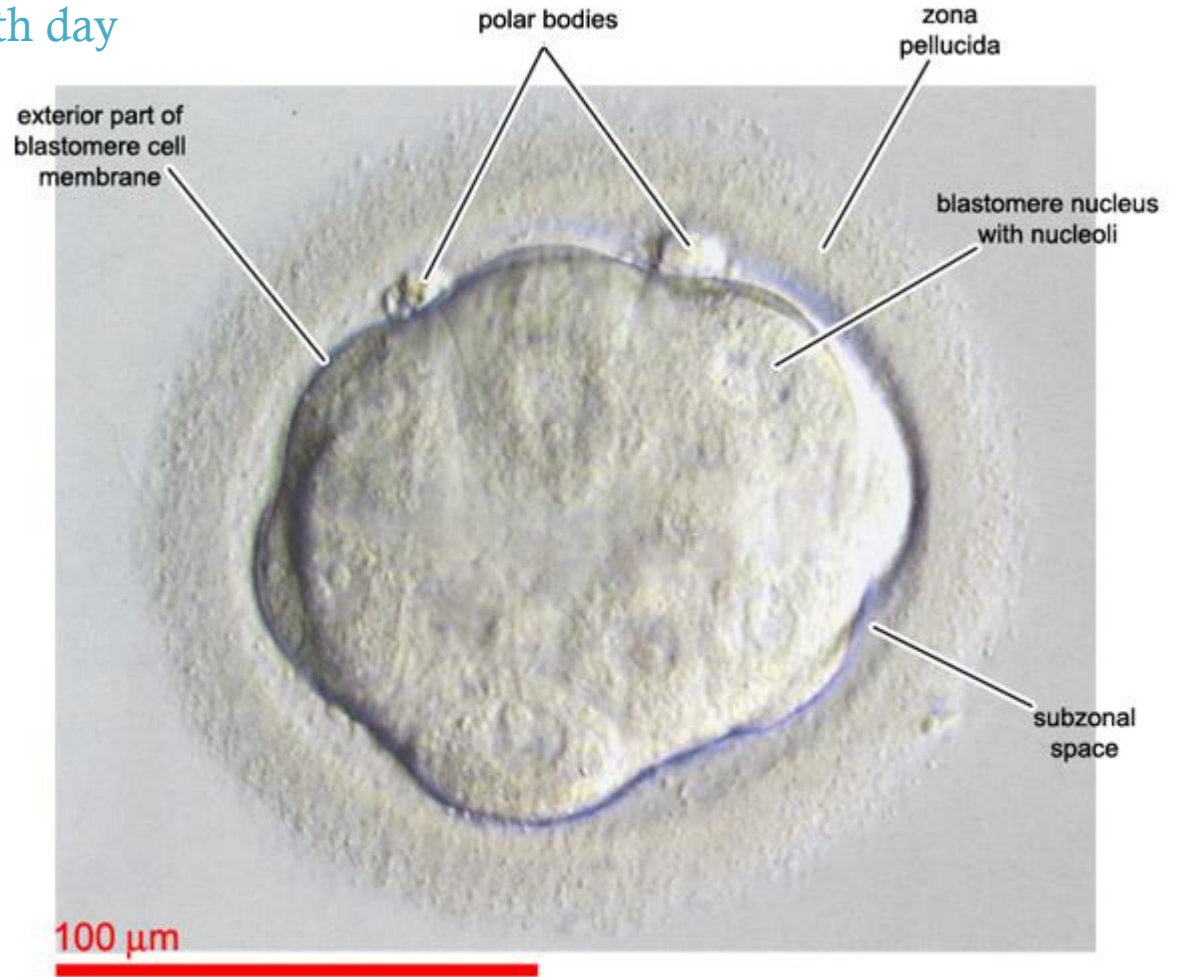


3rd day



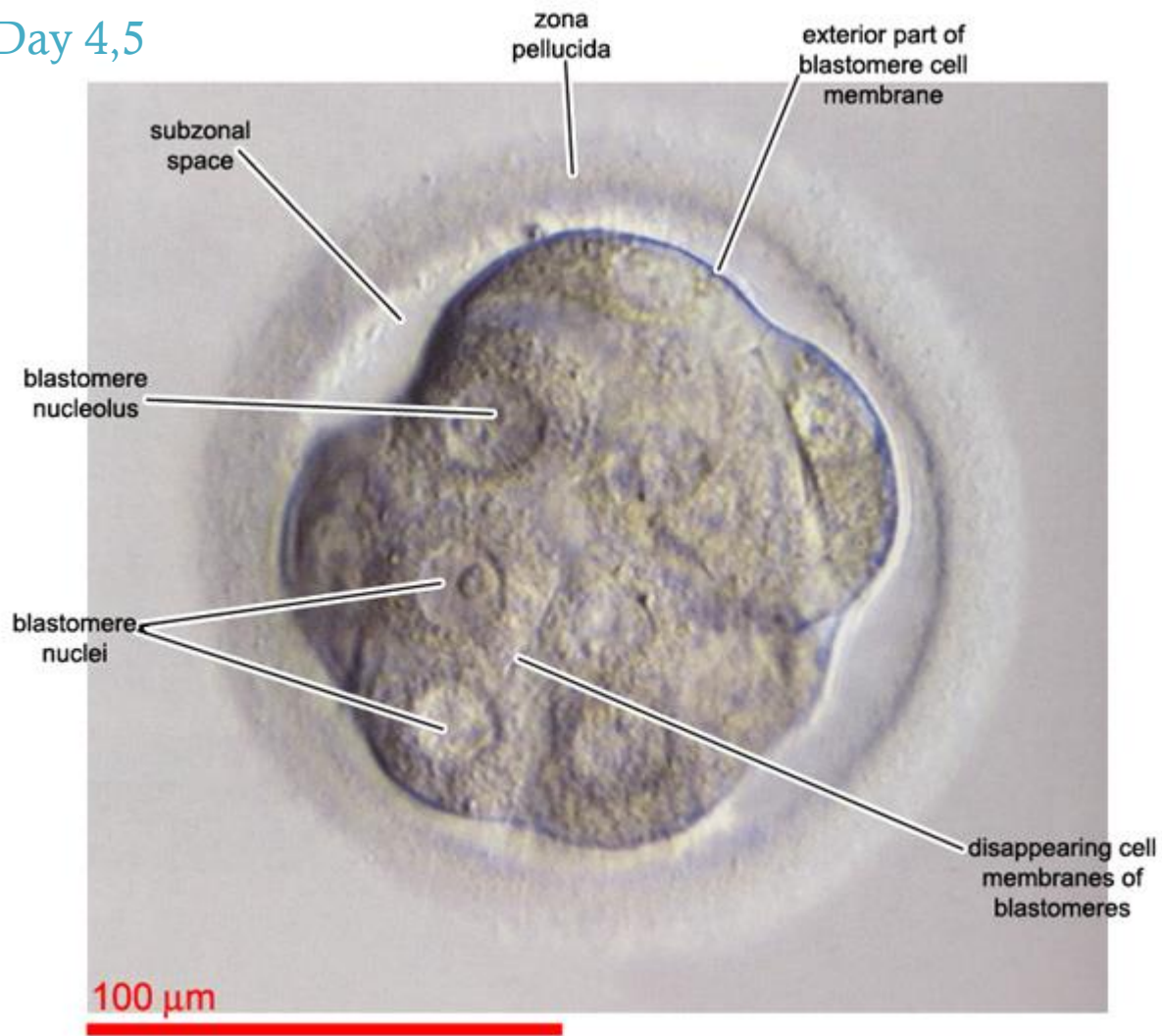
Activation of the embryonic genome

4th day

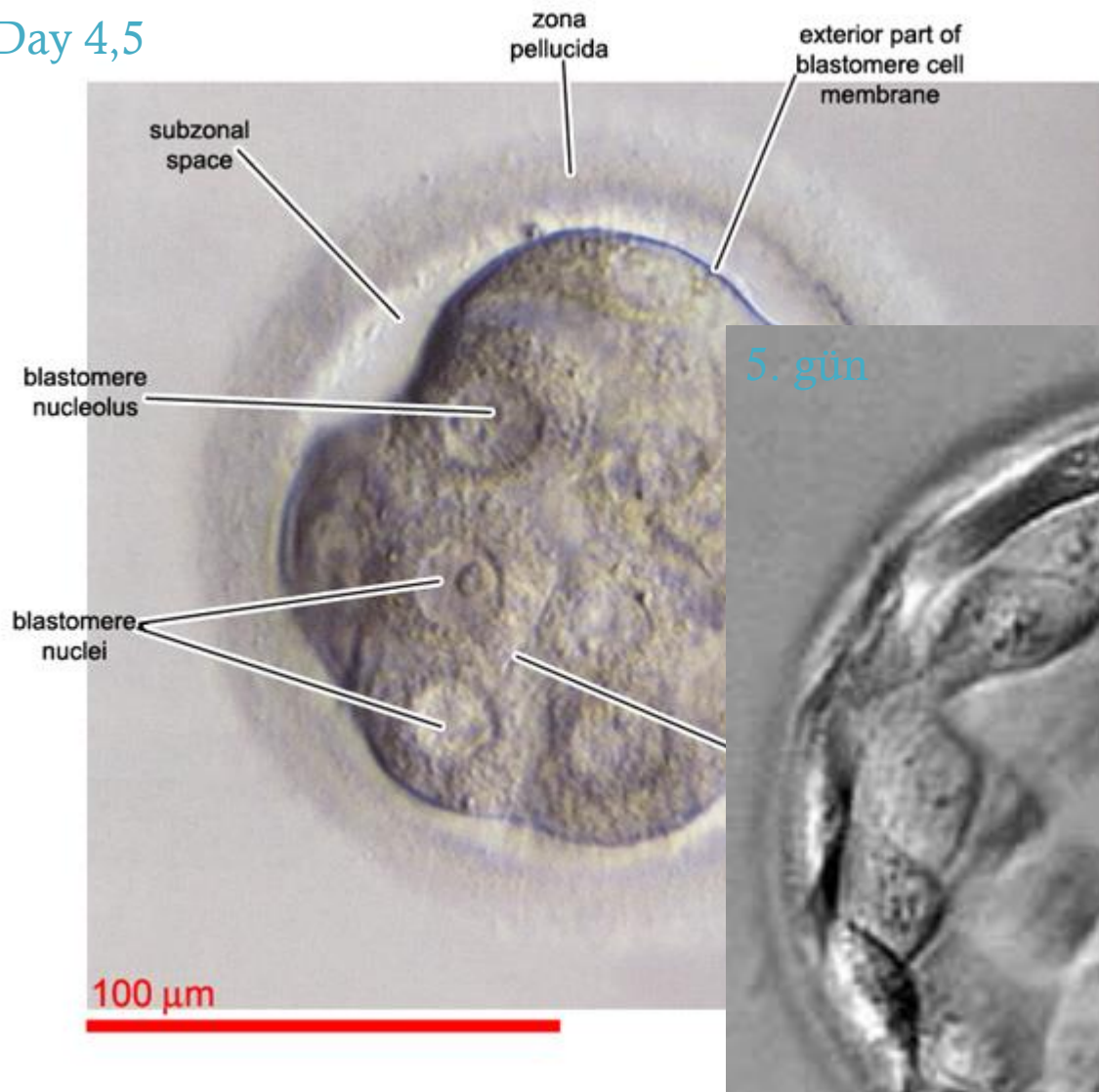


May the 4th be with you!

Day 4,5



Day 4,5



5. gün





Time-lapse microscopy



Hatching blastocyst

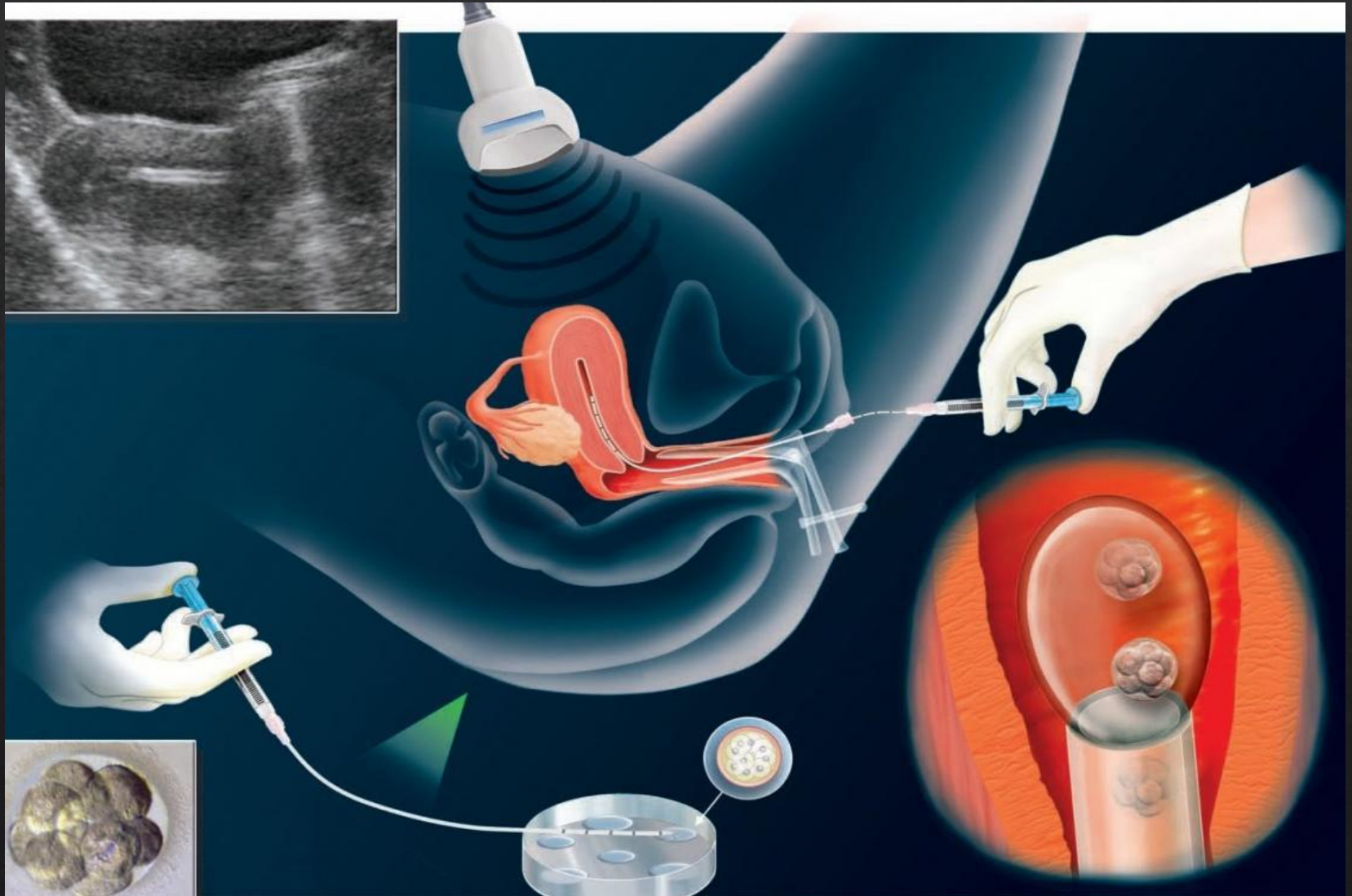
7th day



Hatched blastocyst

Embryo transfer

Pregnancy (b-hCG) test in 10-12 days



Pre-implantation genetic test (PGT)



PGT-A for aneuploidies

PGT-M for monogenic/single gene disorders

PGT-SM for chromosome structural rearrangements

- FISH, CGH, PCR, NGS methods
- Recurrent miscarriages
- Known genetic diseases
- Advanced female age
- HLA compatible bone marrow donor sibling

Cryopreservation

- freezing of gamete-gonads and embryos in liquid nitrogen at -196 degrees.
- Theoretically, the storage period in nitrogen is unlimited as long as it is stored under stable conditions.
- Embryos that are not transferred at the end of treatments (surplus embryos).
- Freezing of all embryos if transfer is canceled for OHSS or other reasons
- Freezing embryos until the results declared after PGT and blastocyst biopsy
- Sperm-oocyte-testicle-ovary freezing for **fertility preservation**



Legal and ethical aspects

Anne ve çocuk sađlığını riske eden çođul gebeliklerin önlenmesi esastır. Bu kapsamda;

a) ÜYTE yöntemlerinden biri olan klasik ovulasyon indüksiyonu ile 2 (iki) den fazla folikül gelişmemesi hedeflenmelidir. Çođul gebeliklerin önlenmesi için üç veya daha fazla folikül gelişmesi halinde artifisyel inseminasyon işlemi yapılması yasaktır.

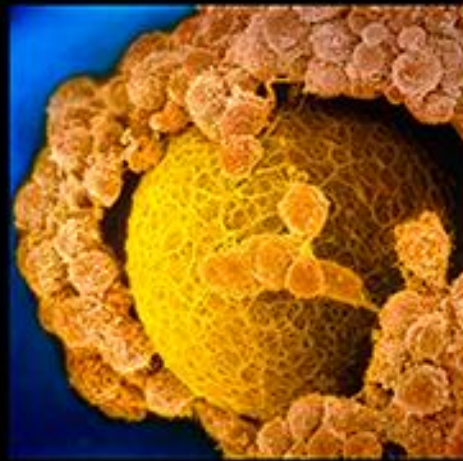
b) Merkezlerde ÜYTE uygulamasında birden fazla embriyo transfer edilmemesi esastır. Ancak, 35 yaşa kadar birinci ve ikinci uygulamada tek embriyo, üçüncü ve sonraki uygulamalarda iki embriyo, 35 yaş ve üzerinde tüm uygulamalarda en fazla iki embriyo transfer edilebilir.

Bu yasaklara aykırı hareket eden merkezler ilk tespitinde 6 ay yeni başvuru kabulü yapamaz, ikinci tespitinde ise merkezin ruhsatı/ faaliyet izni iptal edilir. Ayrıca ÜYTE ünite sorumlusu ve laboratuvar sorumlusunun sertifikası iptal edilir ve bu kişiler merkezlerde çalışamaz.

Legal and ethical aspects

Cinsiyetle ilgili ciddi bir kalıtsal hastalıktan kaçma hali hariç, doğacak çocuğun cinsiyetini belirleme amaçlı gonad ve/veya embriyo seçimi ve transferi yapılamaz. Bu durumun tespiti halinde merkezin ruhsatı/faaliyet izni ile ÜYTE ünite sorumlusunun ve ÜYTE laboratuvar sorumlusunun sertifikası iptal edilir ve bu kişiler merkezlerde çalışamaz.

- Only married couples are accepted for IVF treatments.
- Any third party other than married couples is prohibited from being involved in the treatment like cell (oocyte, sperm, embryo) donation or surrogacy motherhood.



THANK YOU

Dr. Sinan Özkavukcu

sinozk@gmail.com