UNIVERSITY OF VETERINARY MEDICINE - BUDAPEST

Biotechnology 1.
Superovulation, embryo recovery and embryo transfer

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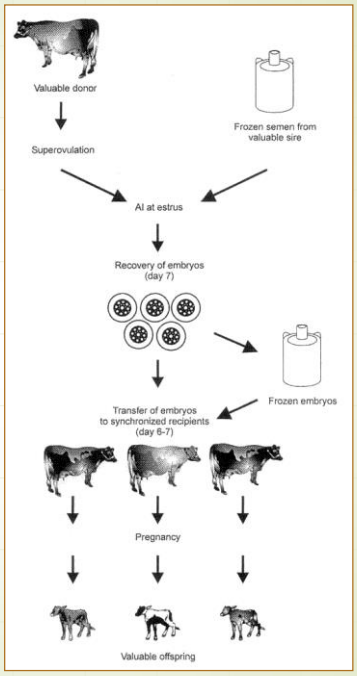
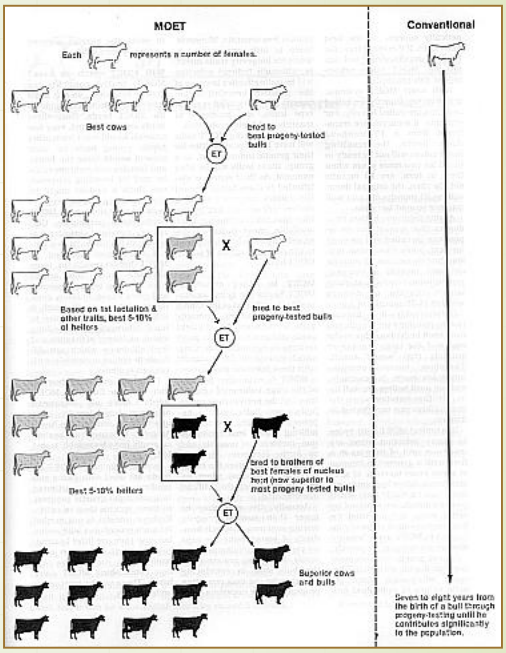
Prof. emeritus

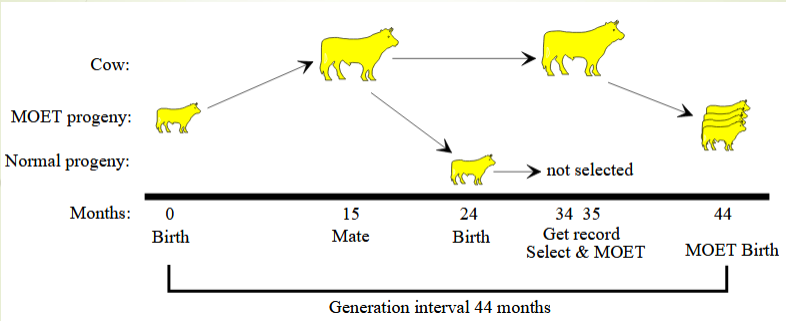
ET flowchart



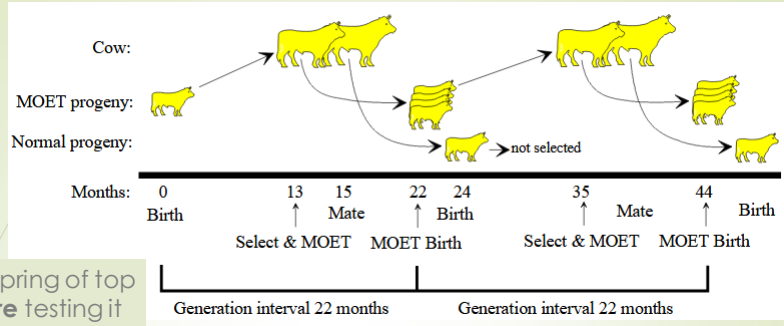
Multiple Ovulation and Embryo Transfer (MOET)



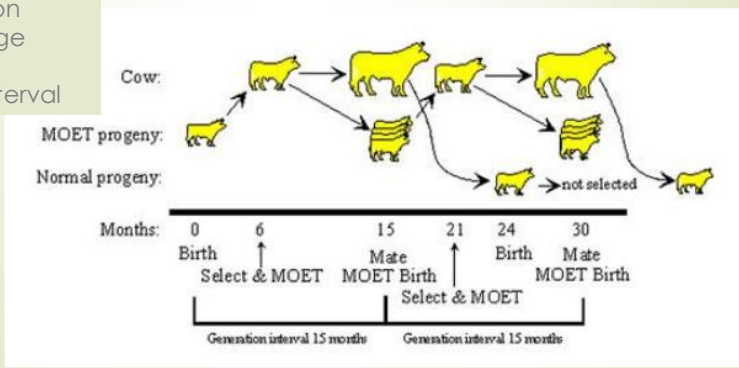
Adult vs juvenile MOET programs



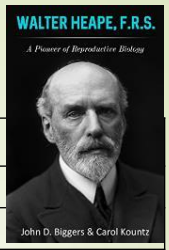
More offspring of top cow **after** testing it
Generation interval 44 months



-More offspring of top cow **before** testing it
-Select base on parent average
-Shortened generation interval



History 1



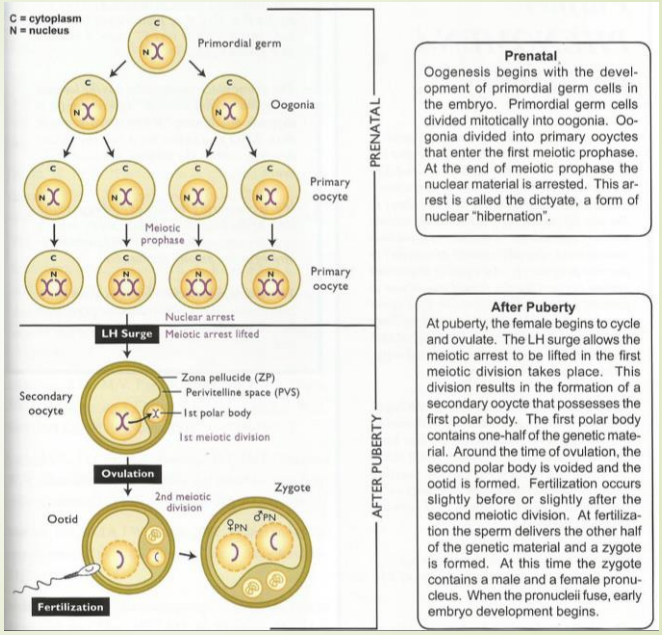
Year	Research group	First successful use
1890	Walter Heape	Embryo transfer in rabbit
1951	Willett et al	Surgical embryo recovery
1964	Sugie	Non surgical embryo recovery (bypass)
1964	Mutter et al	Non surgical (transcervical) transfer
1969	Rowson et al	Surgical recovery and transfer (high success rate)
1972	Sugie et al	Non surgical recovery by Foley catheter
1973	Wilmut and Rowson	Embryo freezing
1981	Willadsen et al	Identical twins by embryo splitting

*Heape, W. (November 1890). "Preliminary note on the transplantation and growth of mammalian ova within a uterine foster-mother". Proceedings of the Royal Society of London. Series B, Containing Papers of a Biological Character. **48**: 457–458. doi:10.1098/rspl.1890.0053.* Heape worked at Cambridge from 1891 to 1906. In November 1897 he published a second paper on his embryo transfer experiments. Heape's contribution to 'applied' science included the rekindling of interest in artificial insemination (1897a, 1898) and the laying of a scientific foundation to the animal breeding industry with emphasis on its economic importance (1899, 1906). In 1906 Heape was elected F.R.S.

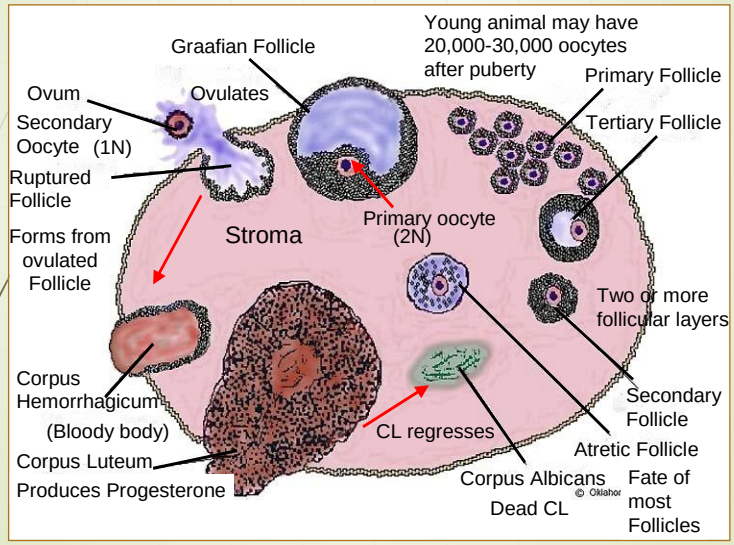
History 2

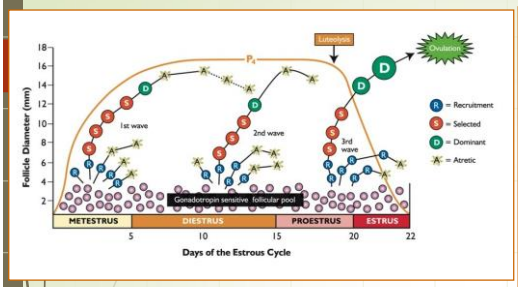
Year	Research group	First successful use
1982	Renard et al	One step freezing method
1983	Lehn-Jensen et al	Freezing of splitted embryos
1983	Brackett et al	In vitro fertilisation (IVF)
1985	Hanada	IVF from slaughterhouse oocytes
1987	Massip et al	Freezing by vitrification
1987	Prather et al	Cloning by nucleus transfer
1990	Herr et al	Sexing of embryos by PCR
1997	Wilmut et al	Somatic cell cloning

Major steps of oogenesis

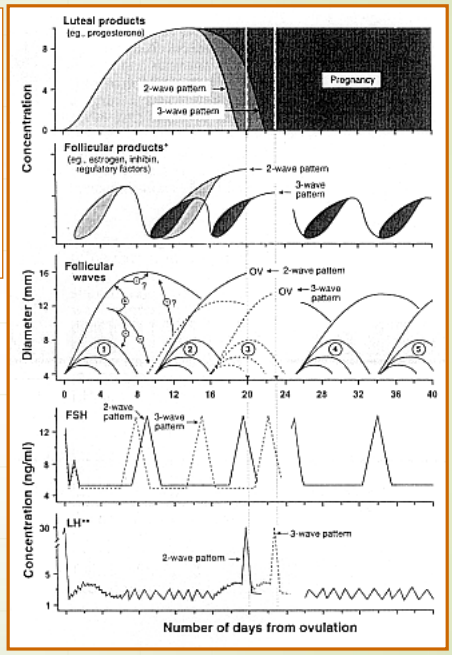


Cross section of the ovary

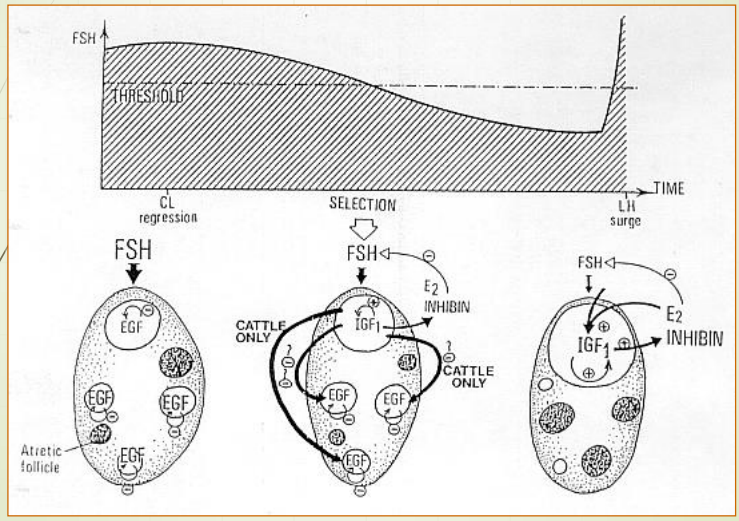




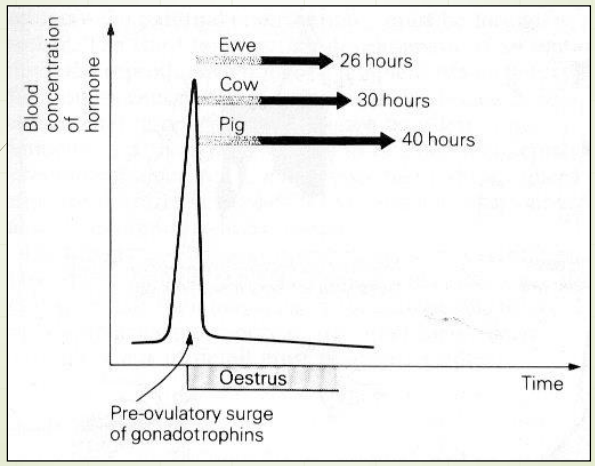
Follicular waves



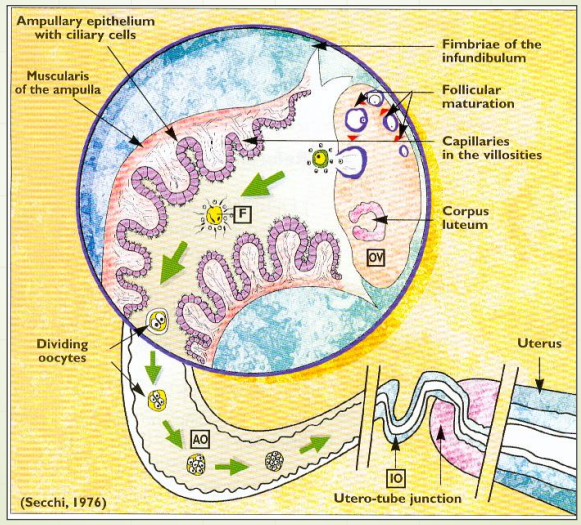
Follicular growth



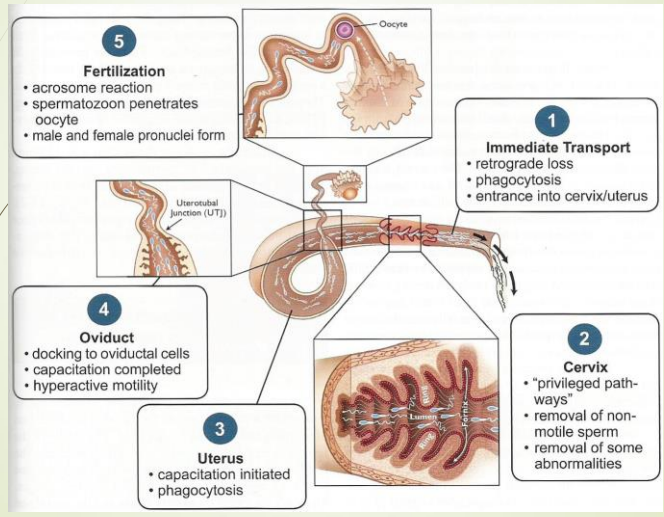
LH peak – ovulation interval



Oviductal transport - fertilization



Events after sperm deposition



Capacitation, acrosome reaction

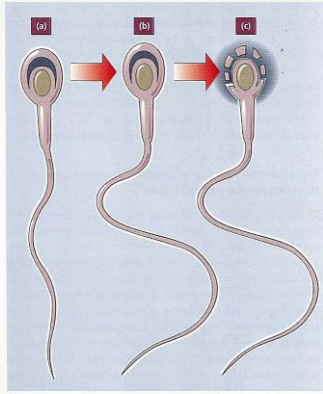
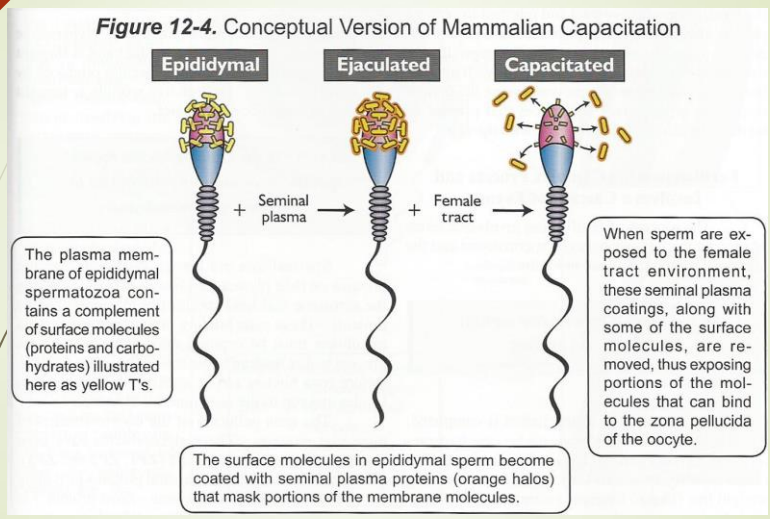
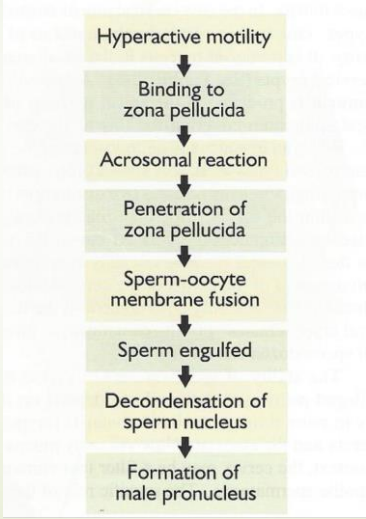


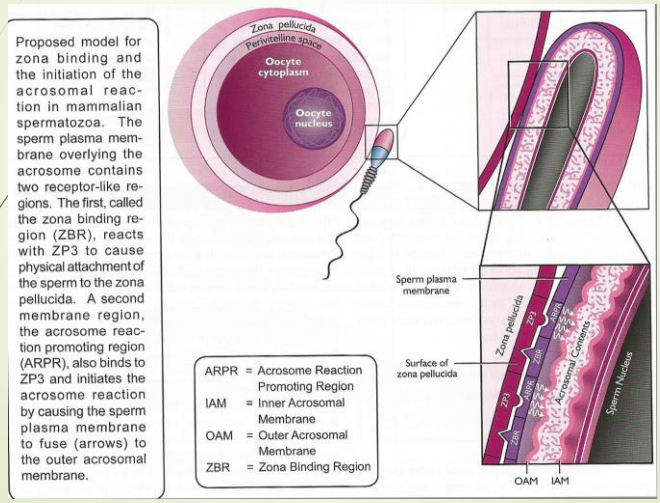
Fig. 9.4 (a) Schematized spermatozoon prior to capacitation; a consequence of capacitation is (b) hyperactivated tail movements, and development of the capacity subsequently to undergo (c) the acrosome reaction, in which multiple sites of fusion between the plasma membrane and the outer acrosomal membrane occur, first at the tip of the acrosome and then at the equatorial region. As a result of the acrosome reaction, the plasma membrane remaining in the equatorial and postacrosomal regions acquires the potential to fuse with the plasma membrane of the oocyte.



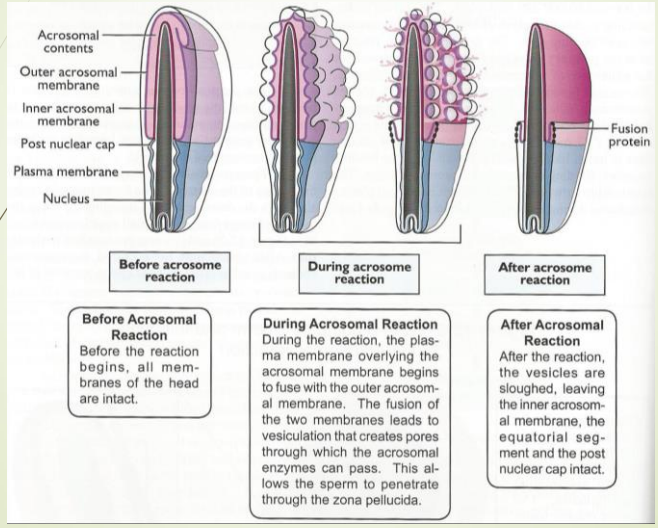
Postcapacitation events and fertilization



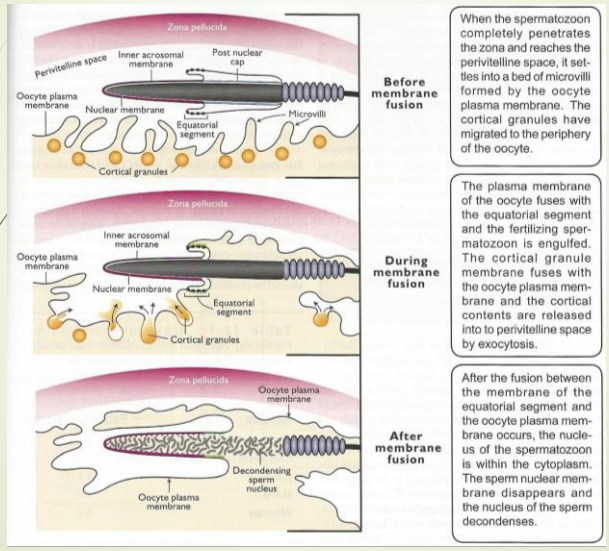
Zona binding and initiation of acrosome reaction



Acrosome reaction



Sperm-oocyte fusion



Activation of Ca^{2+} waves in the oocyte

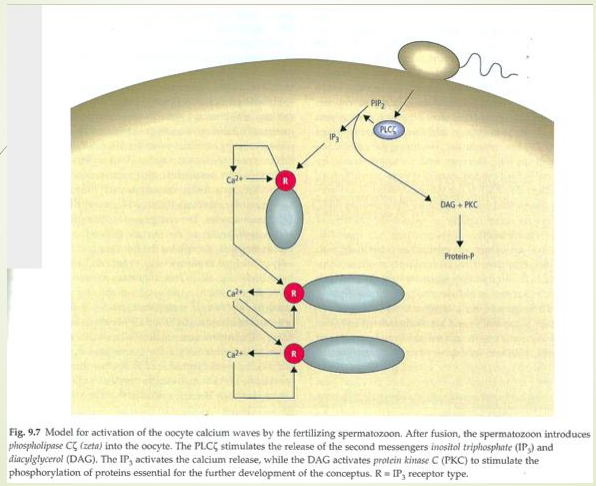


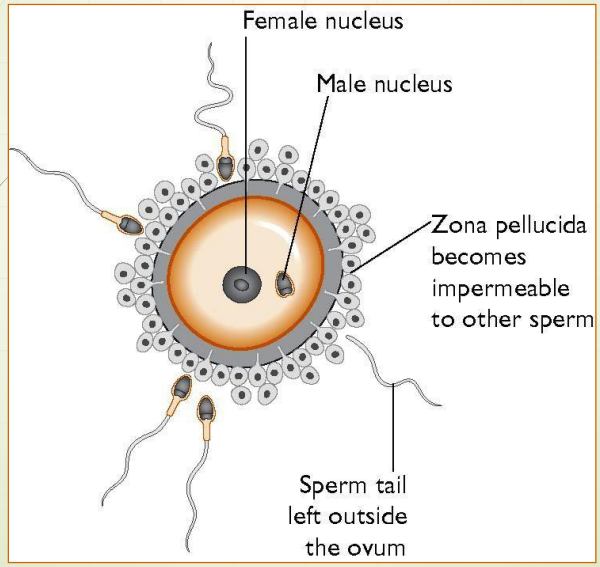
Fig. 9.7 Model for activation of the oocyte calcium waves by the fertilizing spermatozoon. After fusion, the spermatozoon introduces *phospholipase Cζ (zeta)* into the oocyte. The PLCζ stimulates the release of the second messengers *inositol triphosphate* (IP_3) and *diacylglycerol* (DAG). The IP_3 activates the calcium release, while the DAG activates *protein kinase C* (PKC) to stimulate the phosphorylation of proteins essential for the further development of the conceptus. R = IP_3 receptor type.

Developmental and maternal events

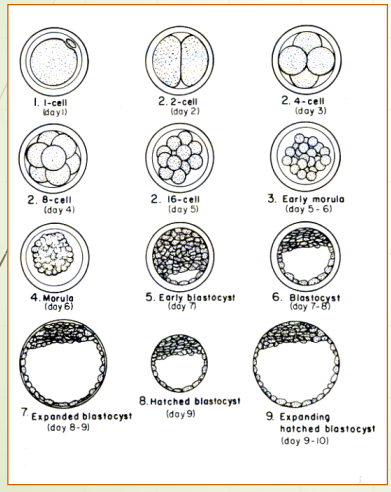
Table 10.1 Times (in days) after ovulation at which various developmental and maternal events occur.

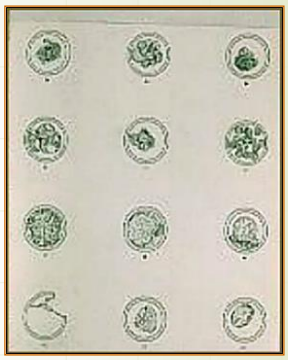
Species	Cleavage to four cells	Major burst of transcription	Conceptus enters uterus	Formation of blastocyst	Time of attachment	Luteal regression time if mating infertile	Duration of pregnancy
Invasive							
Mouse	1.5–2	2-cell	3	3	4.5	10–12	19–20
Rat	2–3	2-cell	3	4.5	4.5–5.5	10–12	21–22
Rabbit	1–1.5	8–16-cell	3.5	3.5	7–8	12	28–31
Human	2	4–8-cell	3.5	4.5	7–9	12–14	270–290
Non-invasive							
Sheep	4	8–16-cell	2–3	6–7	15–16	16–18	144–152
Pig	1–3	4-cell	2	5–6	18	16–18	112–115
Cow	2–3	8–16-cell	3–4	7–8	30–45	18–20	277–290
Horse	1.5–2	?	5–6	6	30–40	20–21	330–345

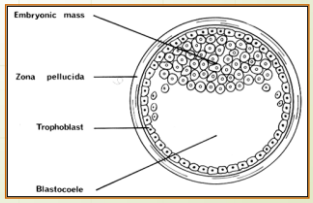
Oviductal transport - fertilization



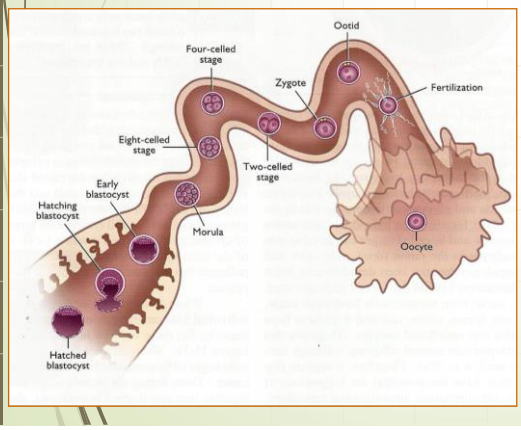
Development of the embryo

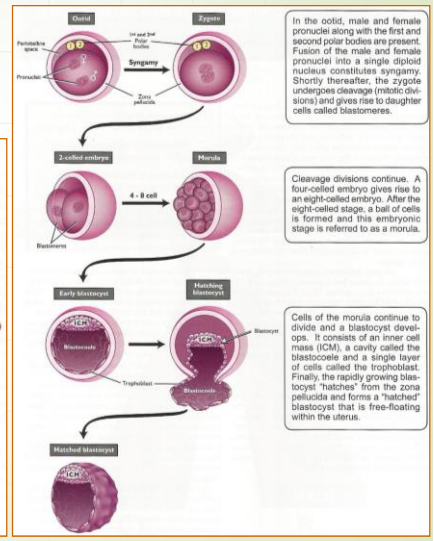






Transport in the oviduct





Embryo compaction and polarization

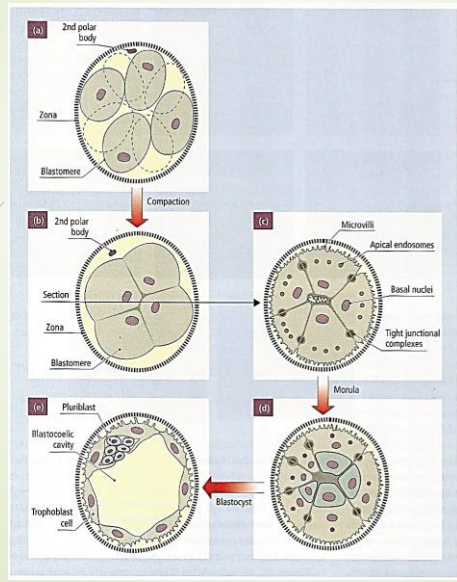
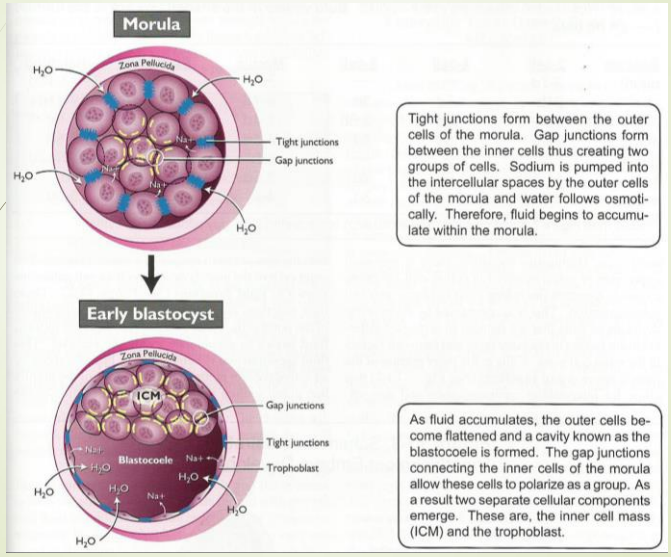
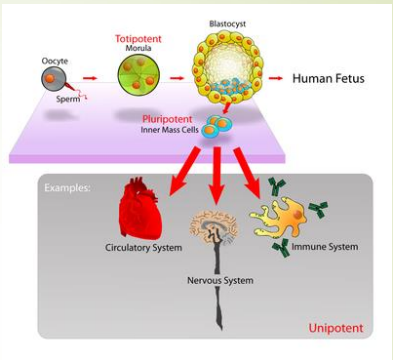


Fig. 10.2 (a–c) Compaction of eight-cell conceptus. Spherical cells (a) become wedge-shaped (b,c) and, by apposing adjacent surfaces, maximize cell contact. In cross-section (c), it can be seen that tight junctional complexes develop between the outer membranes of adjacent cells; these are punctate at first, but later become zonular, forming a barrier to intercellular diffusion between the inside and outside of the conceptus. Each cell also becomes polarized: the nucleus occupying a more basal position, endosomes and other organelles being apical and microvilli being restricted to the exposed surface and points of contact with other cells basally. (d) During cell division to the 16- and 32-cell stages (shown in section), two populations of cells form: the precursors of the outer trophoblast and inner pluriblast (blue) cells. The numbers of each cell type forming depend upon the orientation of the cleavage plane in each cell as indicated. (e) Section through a 64-cell blastocyst; fluid accumulation within the blastocoelic cavity becomes possible when the tight junctional complexes between adjacent trophoblast cells become zonular and prevent its escape. Note the eccentric position of the pluriblast or inner cell mass.

Morula – blastocyst transition



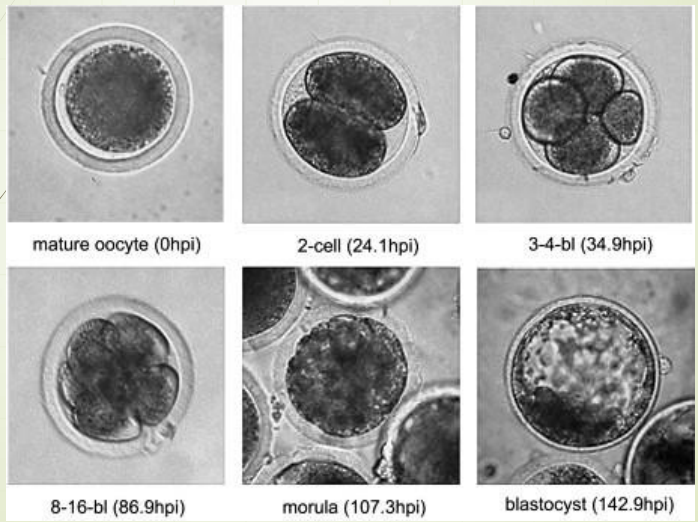
Toti-, pluri- vs. unipotency



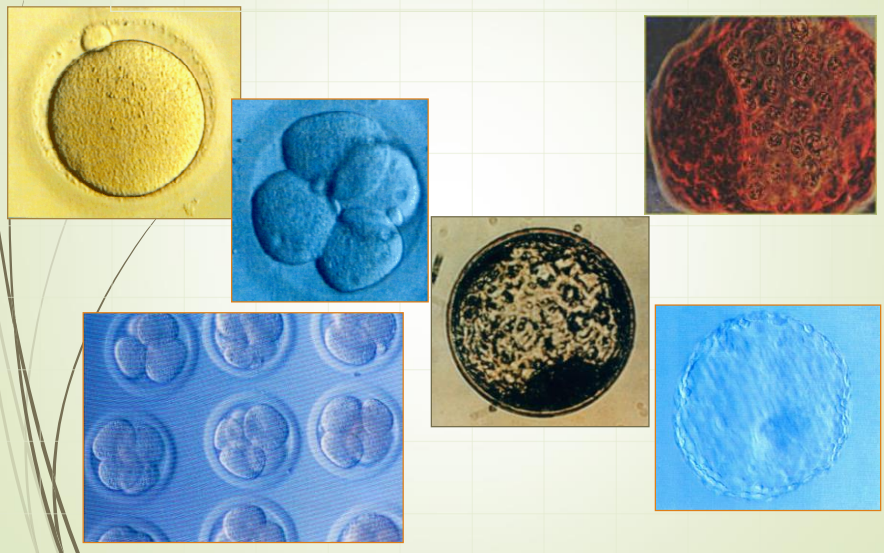
Pluripotent embryonic stem cells originate as inner mass cells within a blastocyst. The stem cells can become any tissue in the body, excluding a placenta. Only the morula's cells are totipotent, able to become all tissues and a placenta

Early embryonic development

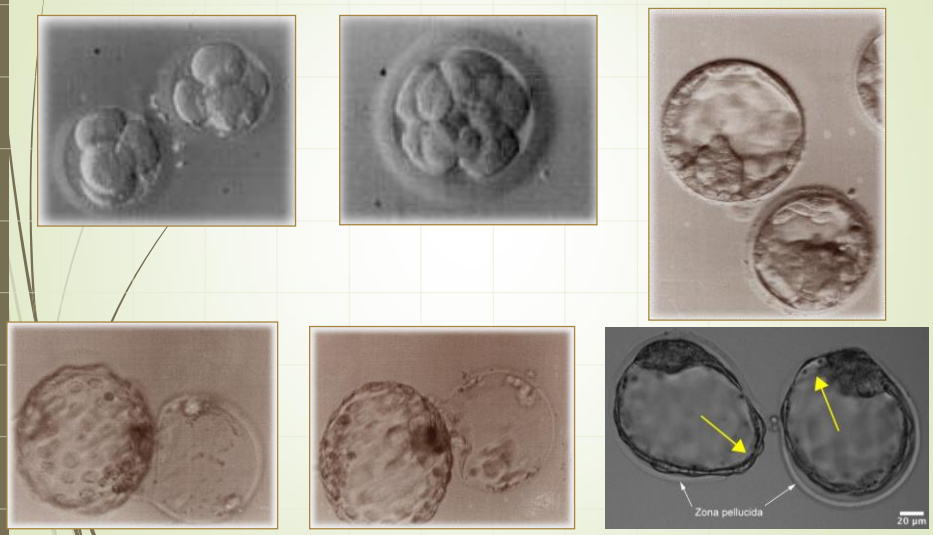
Early embryonic development



Early embryonic development



Embryo photos



Development of human embryos

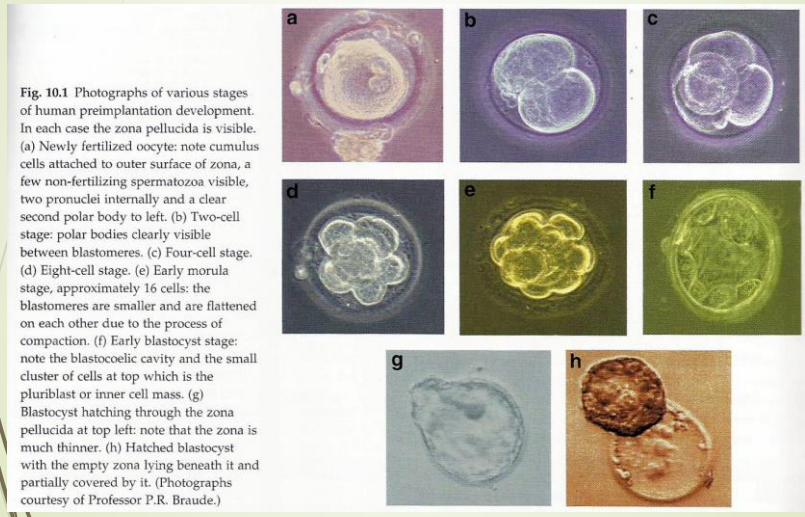
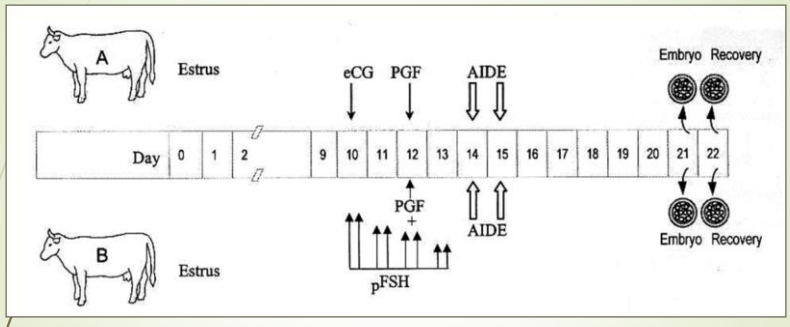
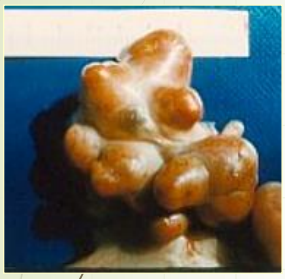


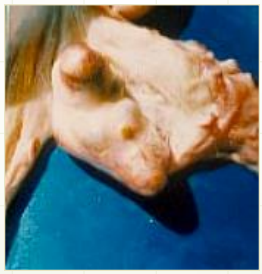
Fig. 10.1 Photographs of various stages of human preimplantation development. In each case the zona pellucida is visible. (a) Newly fertilized oocyte: note cumulus cells attached to outer surface of zona, a few non-fertilizing spermatozoa visible, two pronuclei internally and a clear second polar body to left. (b) Two-cell stage: polar bodies clearly visible between blastomeres. (c) Four-cell stage. (d) Eight-cell stage. (e) Early morula stage, approximately 16 cells: the blastomeres are smaller and are flattened on each other due to the process of compaction. (f) Early blastocyst stage: note the blastocoelic cavity and the small cluster of cells at top which is the pluriblast or inner cell mass. (g) Blastocyst hatching through the zona pellucida at top left: note that the zona is much thinner. (h) Hatched blastocyst with the empty zona lying beneath it and partially covered by it. (Photographs courtesy of Professor P.R. Braude.)

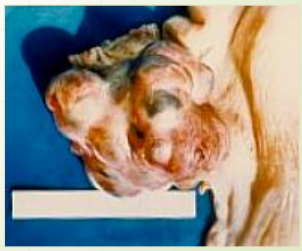
Superovulatory protocols



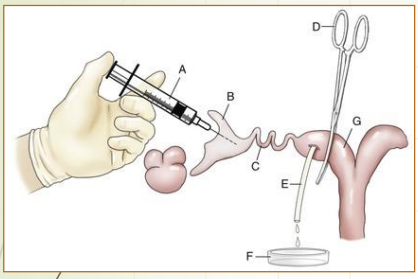
Superovulatory responses

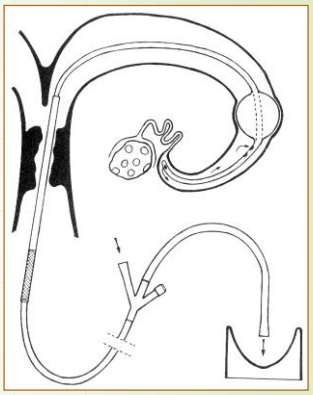


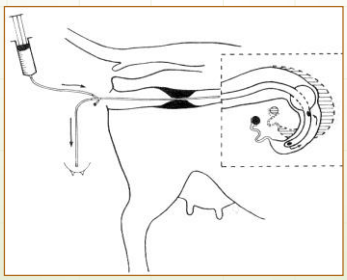




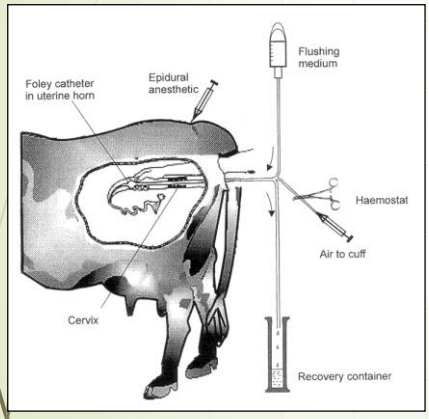
Embryo recovery

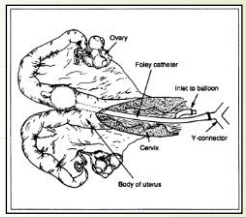


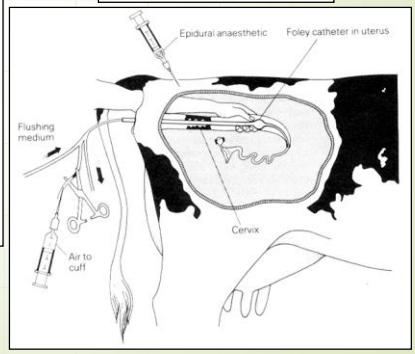




Non-surgical embryo flushing







Recommended culture conditions

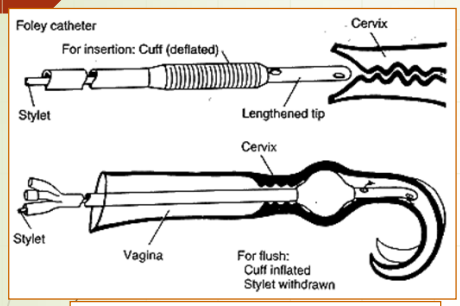
pH	7.2–7.6
Osmolality	270–310 mOsM/kg
Humidity	100 percent
Temperature	Room temperature (15–25°C) or 37°C in incubator
Buffer	Phosphate or bicarbonate ion (latter must be maintained under 5 percent CO_2 atmosphere)*
Sterilization	Filtration of medium through 0.22-µm-pore membranes, aseptic techniques; sterile equipment; addition of 100 IU penicillin G, and 50µg streptomycin sulphate per ml, or 25 µg/ml gentomycin sulfate; addition of antimycotics sometimes indicated
Macromolecule	Sterilized, heat-inactivated serum or serum albumin (e.g. Fraction V, bovine serum albumin)

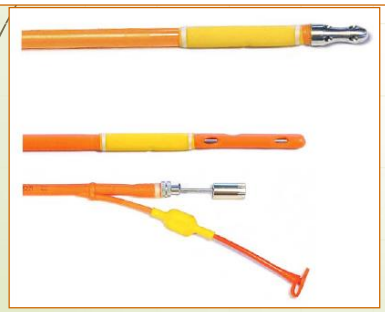
* There is anecdotal evidence that HEPES buffer is detrimental to bovine embryos

Modified Dulbecco's PBS (10 l)

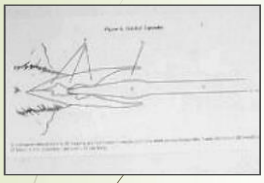
Mixture One	Amount	Function
$CaCl_2.2H_2O$	1.32 g	Membrane/enzyme function
$MgSO_4.7H_2$	1.21 g	Membrane/enzyme function
The above may be weighted in advance and stored indefinitely in a sterile bottle under refrigeration		
Mixture Two	**Amount**	**Function**
NaCl	80.0 g	Osmotic balance; neutralize charge cell membrane
KCl	2.0 g	
Na_2HPO_4	11.5 g	Buffer to maintain pH
KH_2PO4_4	2.0 g	Buffer to maintain pH
Glucose	10.0 g	Energy source
Na pyruvate	0.36 g	Energy source
Streptomycin sulfate	0.5 g	Prevent growth of microorganisms
Na penicillin G	1 000 000 units	Prevent growth of micro-organisms
Mixture Two may be weighed in advance and stored dry in a sterile bottle under refrigeration for six months		
Combination of mixtures One and Two		
Dissolve the reagents in mixture Two in 8 litres of deionized or distilled water. Dissolve mixture One in 2 litres of deionized or distilled water. Add these 2 litres to the 8 litres *stirring constantly*. Other methods of dissolving these ingredients often result in the formation of a precipitate. Sterilize medium by passage through a 0.22-µm bacteriological filter.		

Foley catheter (Rüsch)

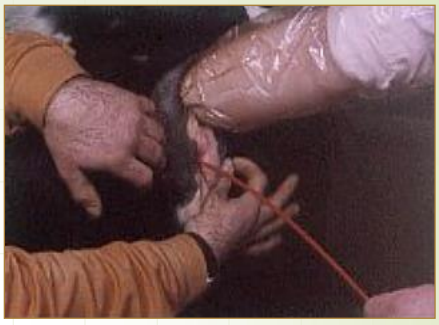




Cervix expander, flushing medium

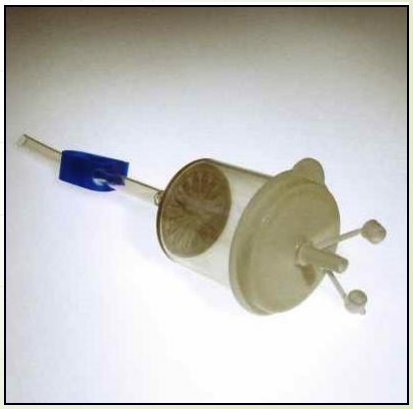


Embryo flushing

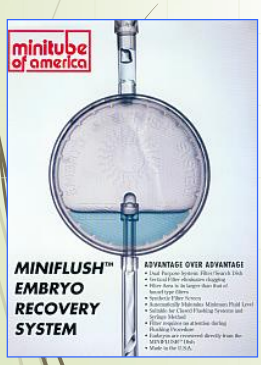


Embryo flushing

Isolation of embryos



Embryo filters



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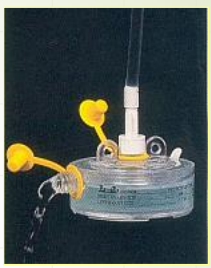
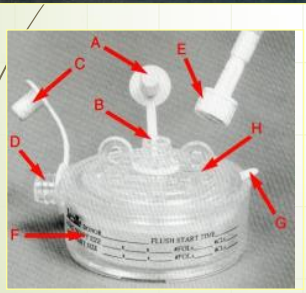
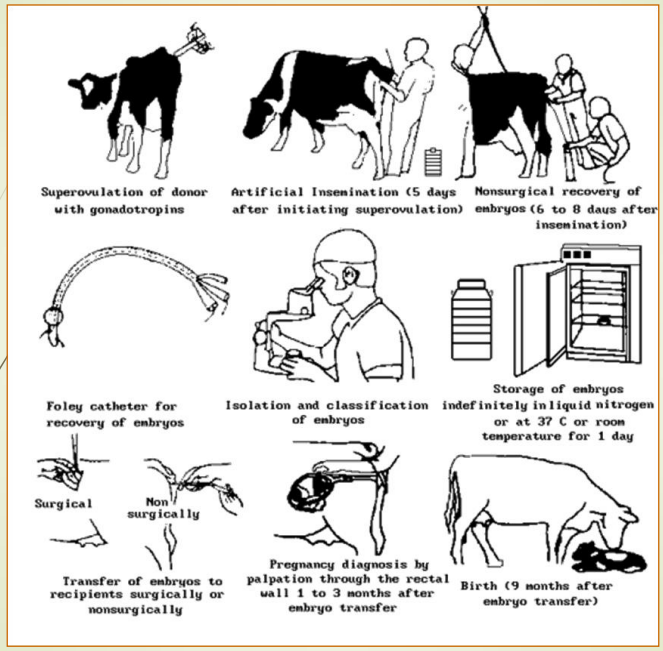






Evaluation of embryos: Stages

(Bó and Mapletoft, 2013)

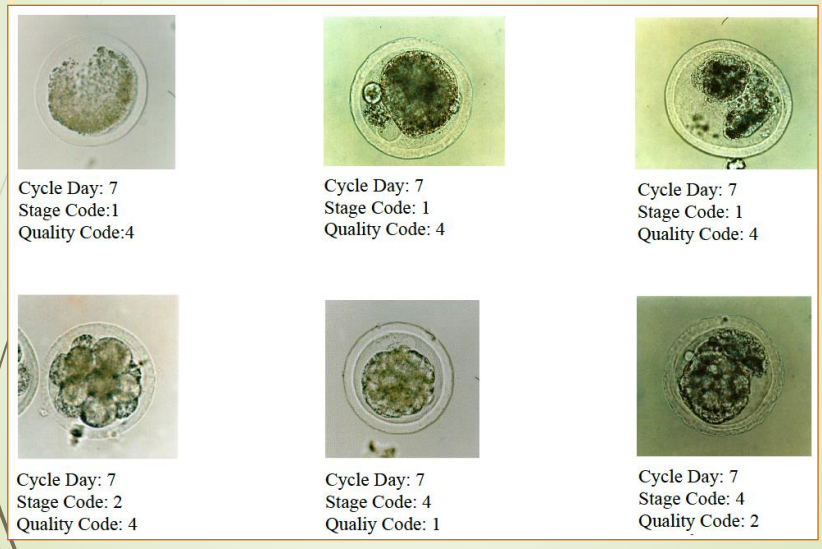
- **Morula (Stage code 3):** A mass of at least 16 cells. Individual blastomeres are difficult to discern from one another. The cellular mass of the embryo occupies most of the perivitelline space.
- **Compact morula (Stage code 4):** Individual blastomeres have coalesced, forming a compact mass. The embryo mass occupies 60 to 70 % of the perivitelline space.
- **Early blastocyst (Stage code 5):** An embryo that has formed a fluid-filled cavity or blastocele and gives a general appearance of a signet ring. The embryo occupies 70 to 80% of the perivitelline space. Early in this stage the embryo may appear of questionable quality because it is difficult to differentiate inner cell mass from trophoblast cells at this time.
- **Blastocyst (Stage code 6):** Pronounced differentiation of the outer trophoblast layer and of the darker, more compact inner cell mass is evident. The blastocele is highly prominent, with the embryo occupying most of the perivitelline space. Visual differentiation between the trophoblast and the inner cell mass is possible at this stage of development.
- **Expanded blastocyst (Stage Code 7):** The overall diameter of the embryo dramatically increases, with a concurrent thinning of the zona pellucida to approximately one-third of its original thickness.
- **Hatched blastocyst (Stage code 8):** Embryos recovered at this developmental stage can be undergoing the process of hatching or may have completely shed the zona pellucida. Hatched blastocysts may be spherical with a well defined blastocele or may be collapsed. Identification of hatched blastocysts can be difficult unless they re-expand when the signet ring appearance is again obvious.

Evaluation of embryos: Quality

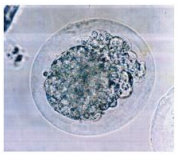
(Bó and Mapletoft, 2013)

- **Code 1: Excellent or Good.** The embryos have a symmetrical and spherical mass with individual blastomeres that are uniform in size, color, and density. This embryo is consistent with its expected stage of development. Irregularities should be relatively minor, and at least 85% of the cellular material should be an intact, viable embryonic mass. This judgment should be based on the percentage of embryonic cells represented by the extruded material in the perivitelline space. The zona pellucida should be smooth and have no concave or flat surfaces that might cause the embryo to adhere to a petri dish or a straw. Code 1 embryos survive well to the freezing/thawing procedure and some practitioners call them "Freezable embryos". Grade 1 embryos are also those recommended for international trade.
- **Code 2: Fair.** These embryos have moderate irregularities in the overall shape of the embryonic mass or in size, color, and density of individual cells. At least 50% of the embryonic mass should be intact. Survival of these embryos to the freezing/thawing procedure is lower than with Grade 1 embryos, but pregnancy rates are adequate if embryos are transferred as fresh into suitable recipients. Therefore these embryos are often called "transferable" but not "freezable".
- **Code 3: Poor.** These embryos have major irregularities in shape of the embryonic mass or in size, color, and density of individual cells. At least 25% of embryo mass must be intact. These embryos do not survive the freezing/thawing procedure and pregnancy rates are lower than those obtained with fair quality embryos if transferred fresh into suitable recipients.
- **Code 4:** Dead or degenerating. These could be embryos, oocytes or 1-cell embryos. They are non-viable and should be discarded.

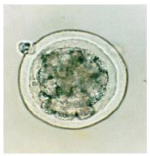
Bovine embryos: developmental stage and quality. Stages 1 to 5



Bovine embryos: developmental stage and quality. Stages 1 to 5



Cycle Day: 7
Stage Code: 4
Quality Code: 2



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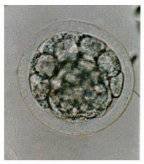

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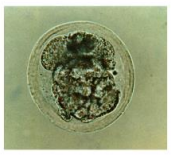

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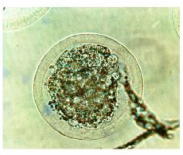

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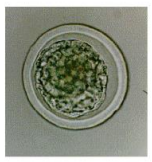

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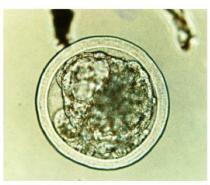
Quality Code: 1

Bovine embryos: developmental stage and quality. Stages 5 to 9



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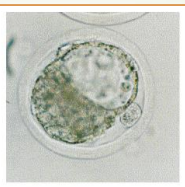
Quality Code: 2



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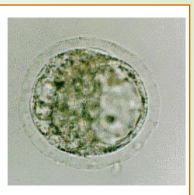

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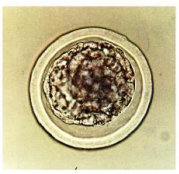

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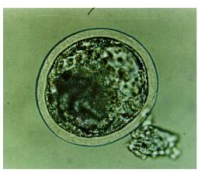

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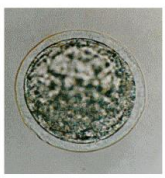

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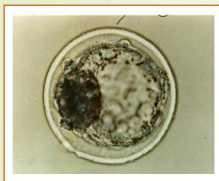
Quality Code: 1

Bovine embryos: developmental stage and quality. Stages 5 to 9



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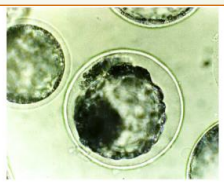
Stage Code: 7
Quality Code: 1



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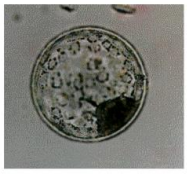

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Stage Code: 7
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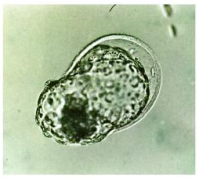

Cycle Day: 8
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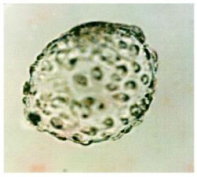

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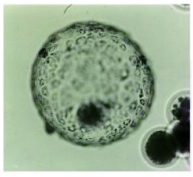
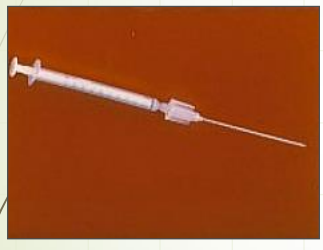
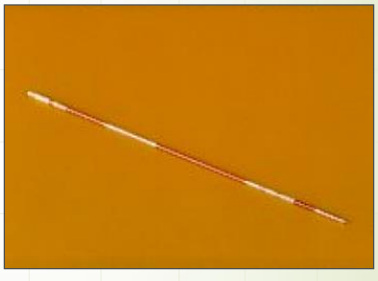
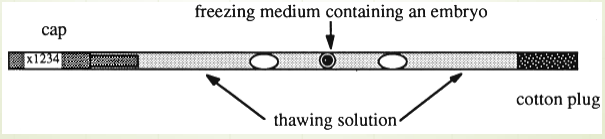


Cycle Day: 9
Stage Code: 9
Quality Code: 1

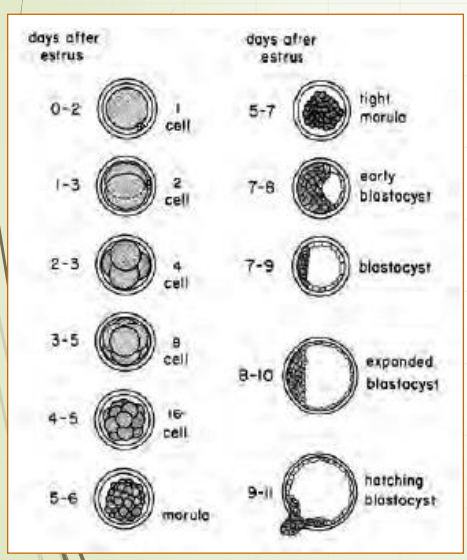
Loading of embryos

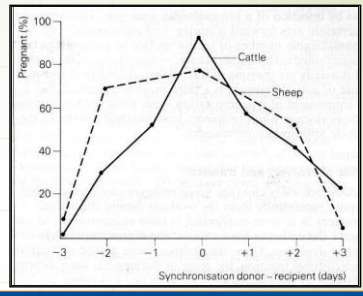


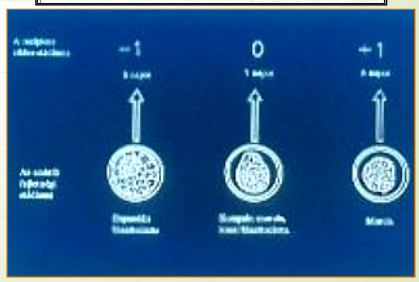


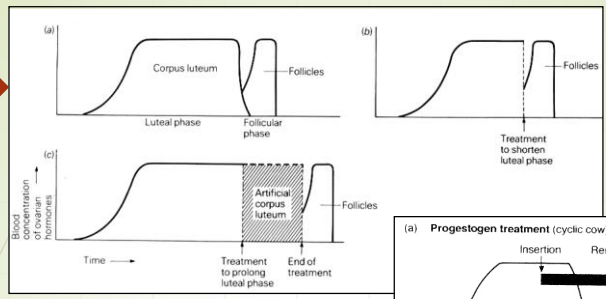


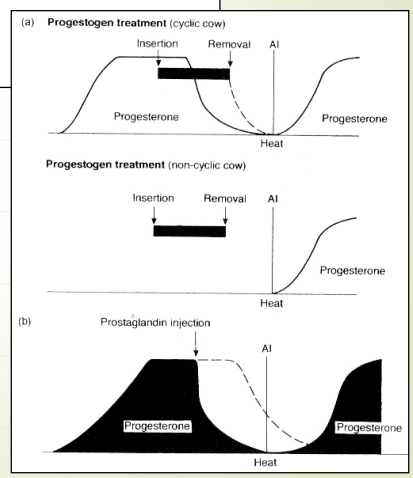
Donor-recipient synchronisation





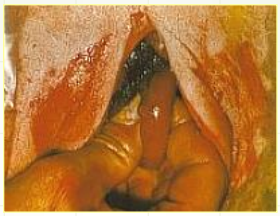
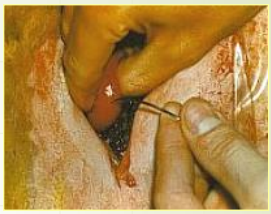
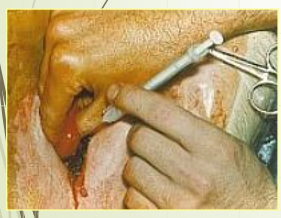
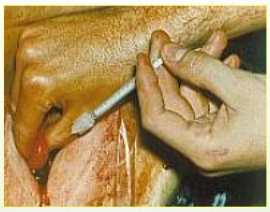




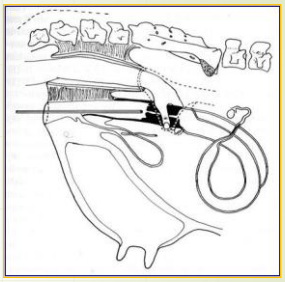
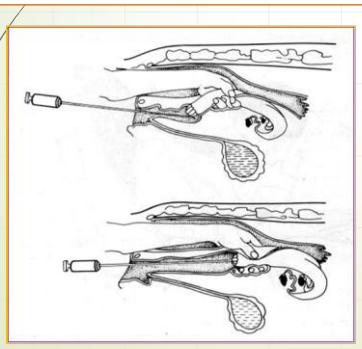


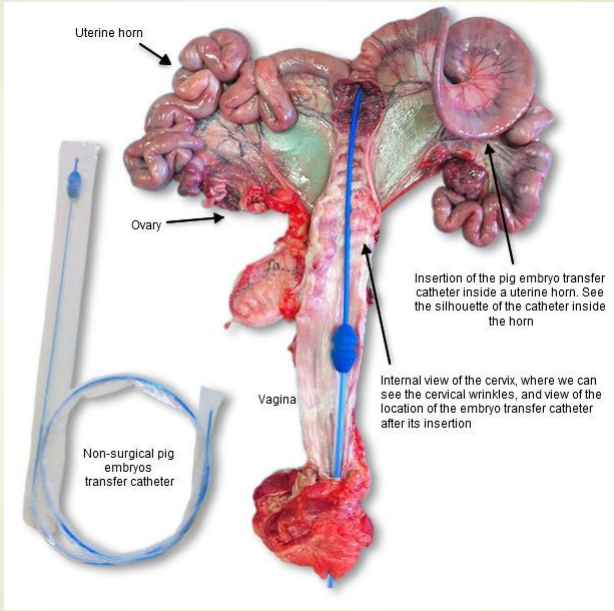
Donor-recipient synchronisation

Surgical transfer of embryos

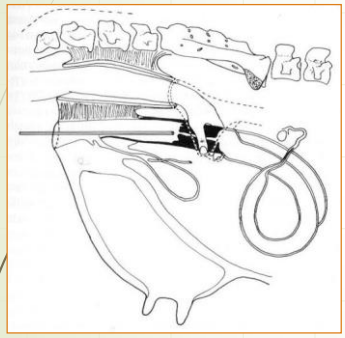






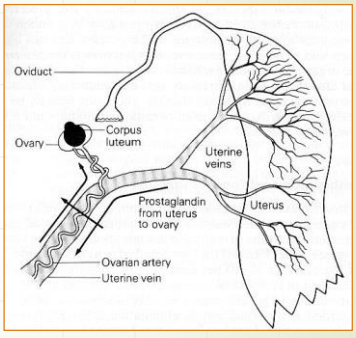

Transfer catheter

Ipsilateral transfer





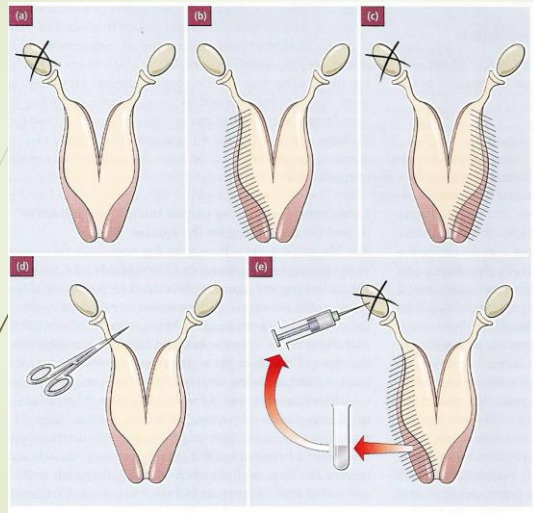


Fig. 5.6 Non-pregnant sheep uterus and ovaries. (a) A single corpus luteum present in the left ovary regresses, indicated by a cross. (b) Removal of the ipsilateral uterine horn (hatched) prevents regression. (c) Removal of the contralateral horn does not prevent regression. (d) Clamping the blood supply between the horn and ovary prevents regression. (e) If the endometrium of the removed ipsilateral horn is homogenized and re-injected into the ovarian artery, the corpus luteum regresses (compare b with e).

Expected Success Rates of ET

Tabelle 1: **Ergebnisse und Veränderungen im OHG-Embryotransfer 2007/08**

Geschäftsjahr	2006/07	2007/08
Durchgeführte Spülungen	178	256
Differenz zum Vorjahr	-22,7 %	+50,6 %
Anzahl der Spendertiere	170	182
Anzahl der ET-Betriebe		88
Gewonnene Embryonen	2089	2852
Durchschnitt/Spülung	12,3	11,1
Transfertaugliche Embryonen	1061	1362
Durchschnitt/Spülung	6,2	5,3
Anteil transfertauglich von gewonnenen Embryonen (%)	50,8	47,8

Tabelle 2: **Verbleib der OHG-Embryonen**

Geschäftsjahr	2006/07	2007/08
Anzahl der frisch übertragenen Embryonen	415	466
davon auf OHG-Trägertiere	237	255
(Anteil gegenüber betriebseigenen Trägern)	51,1 %	54,7 %
Trächtigkeitsrate (%)	51,4	48,2
Anzahl tiefgefrorener Embryonen	658	903
Trächtigkeitsrate bei OHG-Trägertieren aus TG-Embryonen (%)	57,7	62,0
Anzahl verkaufter Embryonen	355	425
Anzahl verkaufter Embryonen/Spülung	2,1	1,7
Durchschnittspreis der Verkaufsembryonen (€)	376	344

(Source: Osnabrücker Schwarzbuntzucht 2009/1)

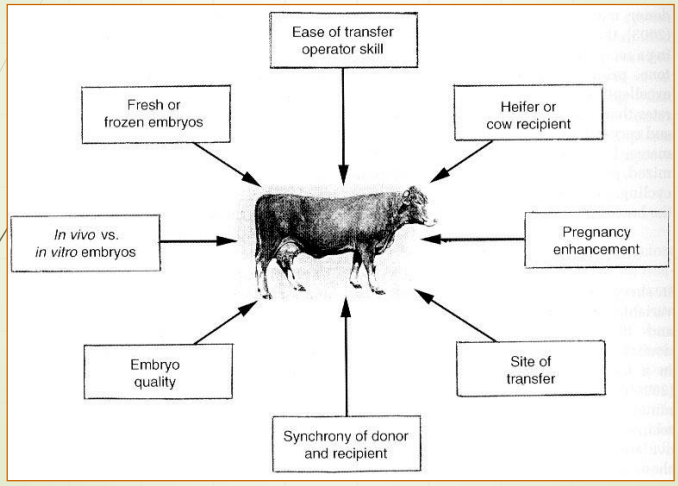
Expected Success Rates of ET

Tabelle 4: **Spendertiere mit den besten ET-Ergebnissen im GJ 2007/08**

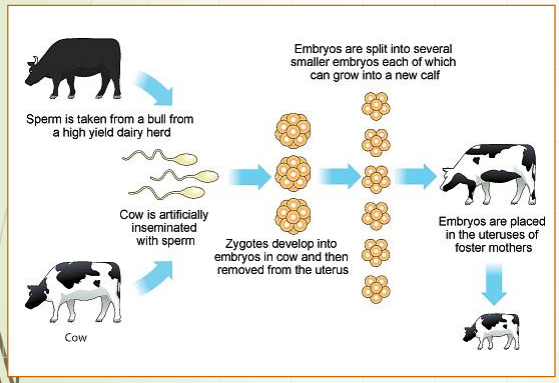
Spendertier: Vater	Besitzer	gefundene Embryonen	transfertaugliche Embryonen	Anpaarungs-bulle
Molly DT Dorado	Niederwestberg, Oberschlochtern	40	30	Virzil
Wonder Red Jordan Red	Gülker, Haldem	26	26	Lawn Boy
Beka FT Lancelot	Pues-Tillkamp, Glandorf	31	21	Bertil
Saint DT Convincer	Niemann, Holzhausen II	28	21	Ralstorm-RF
Dana Ramos	Westrup-Koch GbR, Linne	26	20	Jelder
Palma Lancelot	Wolke, Hartlage	22	19	Eleve
Venedig-Red FT Talent	Niermann, Schiplage	20	19	Ralstorm-RF
Wabe Origin	Wischmeier, Föckinghausen	24	18	Classic PS

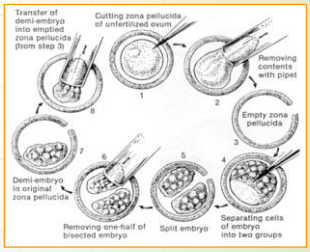
(Source: Osnabrücker Schwarzbuntzucht 2009/1)

Factors influencing the ET



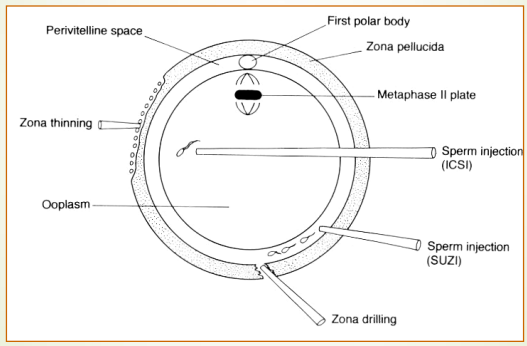
Splitting of embryos (cloning)



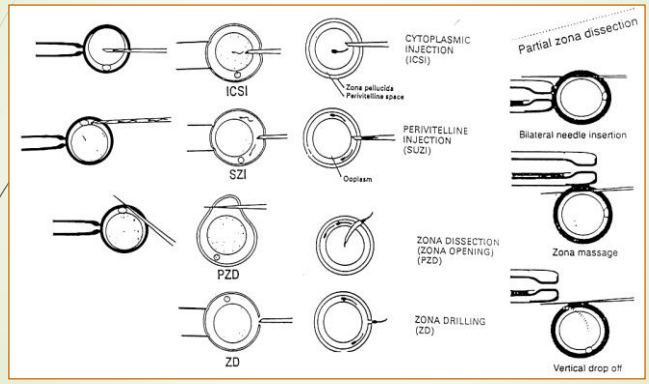


Assisted reproductive technologies – additional methods

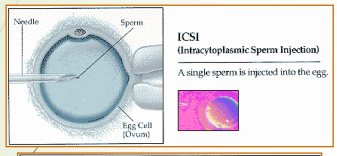
Assisted reproductive techniques (ART)

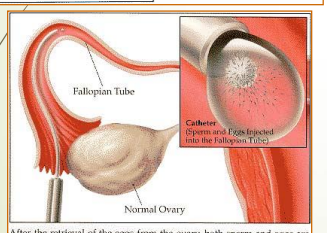


Assisted reproductive techniques (ART)

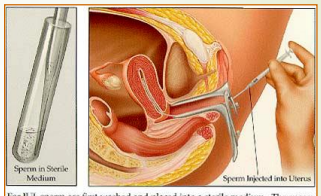


ART

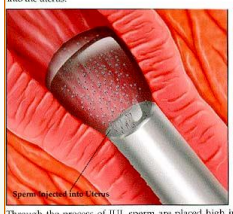




After the retrieval of the eggs from the ovary, both sperm and eggs are injected through the catheter directly into the fallopian tube. Fertilization may then take place normally in the fallopian tube.

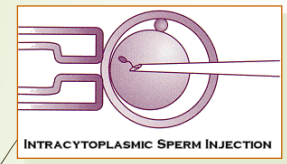


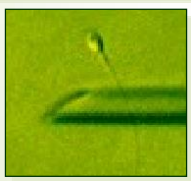
For IUI, sperm are first washed and placed into a sterile medium. The sperm are then concentrated in a small volume of medium and are injected directly into the uterus.



Through the process of IUI, sperm are placed high in the female reproductive tract to enhance the chance of successful fertilization.

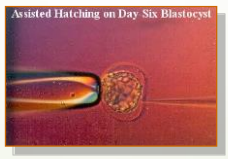
Microfertilization (ICSI, SUZI)





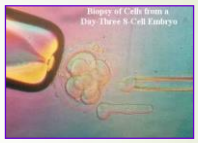
Assisted hatching



Embryo biopsy - prenatal diagnostics





Preimplantation Genetic Diagnosis (PGD)

Definition: A process which allows parents to have the option of detecting potential defects in an embryo within days after conception