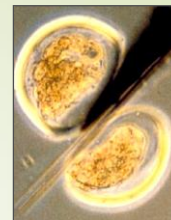


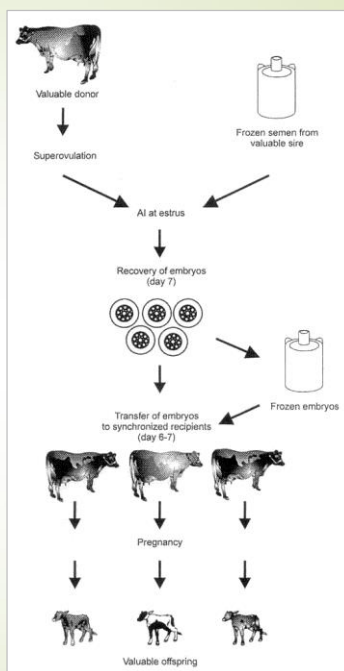
ÁLLATORVOS-TUDOMÁNYI EGYETEM, BUDAPEST



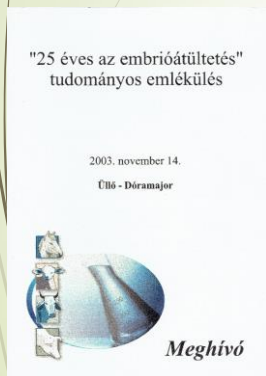
## Biotechnológia 1. Szuperovuláció, embriók kinyerése és beültetése

Solti László  
Prof. emeritus

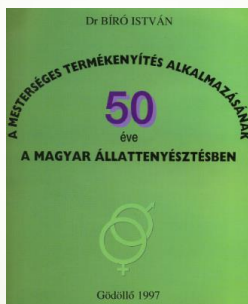
ET  
folyamatábra



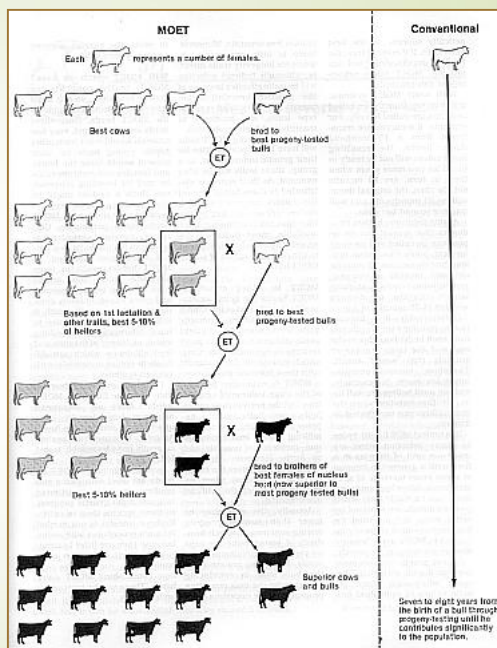
# Évfordulók: 50 éves az MT (1997) 70 éves az MT és 40 éves az ET (2017)



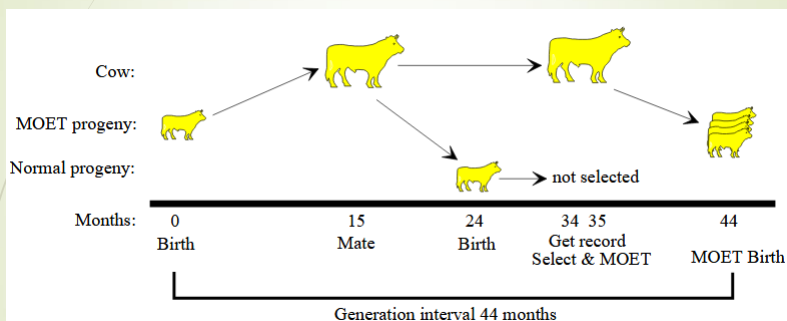
NESZMÉLYI K, FLINK F: 25 éves az embrióátültetés.  
Magy. Áo. Lapja, 2004. 126. 252-253.



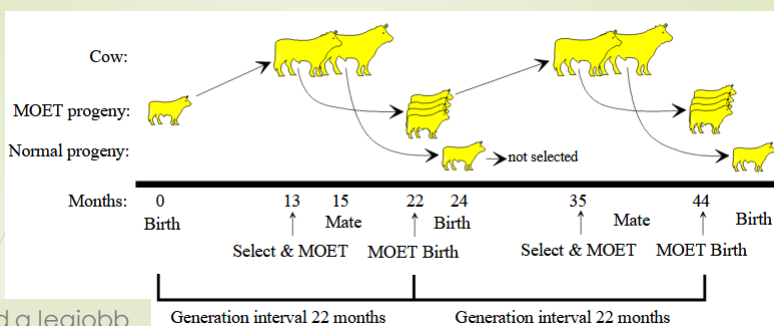
## Multiple Ovulation and Embryo Transfer (MOET)



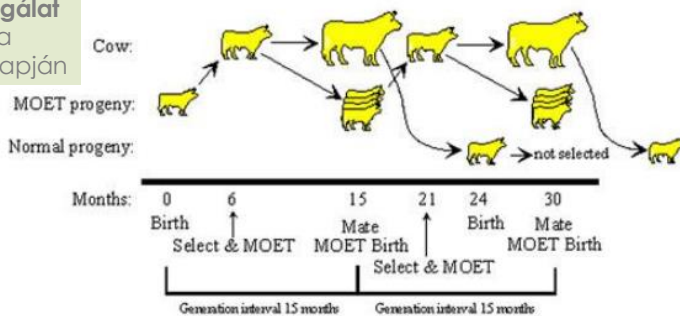
## Adult és juvenilis MOET program



Több utód a legjobb tehenektől  
teljesítményvizsgálat után



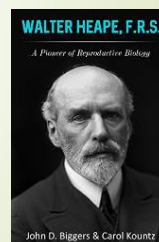
Több utód a legjobb tehenektől  
teljesítményvizsgálat előtt. Szelekció a szülők átlaga alapján



## Az ET mérföldkövei

Év	Kutatócsoport	Az első sikeres alkalmazás
1890	Walter Heape	Embrióátültetés nyúlban
1951	Willet és mtsai	Sebészi embriókinyerés
1964	Sugie	Nem sebészi embriókinyerés (bypass)
1964	Mutter és mtsai	Vértelen (transzcervikális) beültetés
1969	Rowson és mtsai	Magas hatékonyságú sebészi átültetés
1972	Sugie és mtsai	Vértelen embriónyerés Foley-katéterrel
1973	Wilmut és Rowson	Embriómélyhűtés
1981	Willadsen és mtsai	Identikus ikrek embriófelezéssel

Év	Kutatócsoport	Az első sikeres alkalmazás
1982	Renard és mtsai	Egylépcsős fagyasztás
1983	Lehn-Jensen és mtsai	Darabolt embriók fagyasztása
1983	Brackett és mtsai	Szma in vitro fertilizáció (IVF)
1985	Hanada	IVF vágóhídi petefészekből
1987	Massip és mtsai	Vitrifikációs mélyhűtés
1987	Prather és mtsai	Klónozás sejtmagátültetéssel
1990	Herr és mtsai	Embrió iv armeghatározás (PCR)
1997	Wilmut és mtsai	Klónozás szomatikus sejtekből



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/rpsl.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.

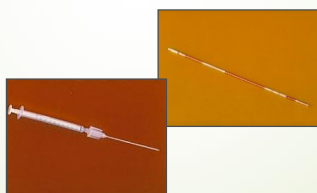
## Történelmi áttekintés (ET)

- 1950-es évek: ET kísérletek nyúlban (Barna és mtsai, AKI)
- 1977: ET kísérletek nyúlban és juhban (Haraszi J, Rónay G)
- 1977 december: megszületett az első ET borjú („Németh Lajos”) import német embrióból → vértelen mosás és beültetés (Joachim & Rudolf Hahn, Mészáros József, Perjés István)
- 1977: kanadai ET bemutató Kiskunfélegyházán, döntés az Üllői Embrióátültető Állomás létrehozásáról (munkacsoport alakítása: Solti L, Mészáros J, Köcsky L, Perjés I, Sándor L), az OÁF üllői bázisán

## Történelmi áttekintés

- ▶ 1978: Embrióátültető Állomás import embriók fogadására, az ET adaptálására, szakember képzésre és embrió termelésre
- 1979-90: 2000 import és 1000 hazai szarvasmarha ET (Üllő)
- 1982-1986: 850 import juh ET (Üllő)
- 80-as évek második fele: üzemi juh ET technológia kialakítása, bevezetése, kb. 500 juh ET üzemekben
- ▶ 1980: szarvasmarha, 1982: juh ET üzemi körülmények között
- ▶ 1983: embrió mélyhűtés
- ▶ Üllő-Gödöllő: génmegőrzési programok ET-vel

## ET életképek – Üllő és ÁOTE

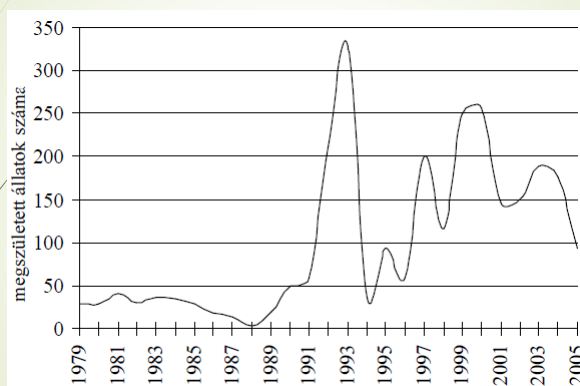


## Történelmi áttekintés



- 80-as évek közepe: Mosonmagyaróvár, Embrióátültető Állomás import Lacaune juhállomány mentesítése ET-vel  
Kiméra báránnyok, juh-kecske kiméra mikrosebészettel (1986)
- 1989: Mezőgazdasági Biotechnológiai Kutatóközpont (Gödöllő)
- IVF laboratórium, az első IVF borjú (Üllővel), magyar szürke IVF borjú
- MBK: Transzgenikus nyulak előállítása (német kollaboráció, G. Brem), PCR ivarmeghatározás embrió biopótumból
- 90-es évek: endoszkópos embrióátültetés sertésen (Rátky és Brüssow)
- 90-es évek közepe: Szarvas sperma tartós tárolás és szarvas embrióátültetés (Kaposvári Egyetem)

## ET-ből született borjak száma



Forrás: Szabari 2008



A Kaposvári Egyetem Embrió Központjának modern laboratóriuma

**EU átlag:** 6.3  
embrió/donor  
(Ausztria: 11.4,  
Olaszország: 8)

**Magyar átlag:** 7.4  
embrió/donor

Szexált spermás MT:  
12 országban

**ET állomások:**

- Embrió Kft. Pécs
- Bonafarm Zrt  
Csípőtelek
- Kaposvári Egyetem
- Debreceni Egyetem  
Karcag (juh/kecske)

Ország	Átültetett embriók						Összesen	Exportált embriók
	In vivo			In vitro				
	Friss	Mélyhűtött hazai	Mélyhűtött külföldi	Friss	Mélyhűtött hazai	Mélyhűtött külföldi		
Ausztria	456	759	28	12	5	0	1260	
Belgium	1275	3869	1007	0	0	0	6151	146
Csehország	0	5	0	0	0	0	5	0
Dánia	2238	1007		0	0	0	3245	91
Finnország	762	1544	496	66	43	0	2911	4
Franciaország	18 837	17 200	1042	251	297	0	37 627	220
Görögország	5	0	25	0	0	0	30	0
Hollandia	4072	16 406	0	7287	899	0	28 664	1115
Irország	720	750	0	0	0	0	1470	0
Lengyelország	608	588	0	0	0	0	1196	0
Litvánia	54	36	0	0	0	0	90	0
Luxemburg	736	791	0	0	45	0	1572	50
<b>Magyarország</b>	<b>289</b>	<b>212</b>	<b>106</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>607</b>	<b>10</b>
Németország	8017	12 546	0	1684	358	0	22 605	0
Norvégia	22	45	55	0	0	0	122	0
Olaszország	6003	n. a.	n. a.	329	1337	0	6003	n. a.
Orosz Föderáció	104	1510	482	0	5	0	2101	0
Portugália	124	450	35	0	0	0	609	16
Spanyolország	869	1130	138	171	76	8	2392	56
Svédország	950	1969	523	0	0	0	3442	72
Svédország	768	329	263	0	0	0	1360	0
Szlovénia	10	2	2	0	0	0	14	0
<b>Összesen</b>	<b>46 919</b>	<b>61 148</b>	<b>4202</b>	<b>9805</b>	<b>2944</b>	<b>8</b>	<b>123 476</b>	<b>1780</b>

<http://magyarmezgazdasag.hu/2016/11/09/hazai-embriok-mihosege-kivalo>

REGION / COUNTRY	BOVINE IN-VIVO DERIVED EMBRYO COLLECTION											
	COLLECTIONS / FLUSHES						UNGRADED EMBRYOS COLLECTED			TRANSFERRABLE EMBRYOS		
	CONVENTIONAL SEMEN			SEXED SEMEN			DAIRY	BEEF	TOTAL	DAIRY	BEEF	TOTAL
Africa		599	597	0	0	0	315	3824	4139	240	3863	4103
South Africa		553	591	0	0	0	315	3784	4099	240	3850	4090
Zimbabwe		6	6	0	0	0	0	40	40	0	13	13
Asia	2426	12391	14817	2	0	2	22933	169063	191996	13226	99372	112598
Israel	38	2	40	2	0	2	265	10	275	182	10	192
Japan	2388	12389	14777	0	0	0	22668	169053	191721	13044	99362	112406
Europe	14677	4675	19352	1338	93	1431	151257	43530	194787	99693	29184	128877
Austria	168	64	232	26	1	27	1836	890	2726	1397	641	2038
Belgium	155	971	1126	8	1	9	1150	6073	7223	694	5059	5753
Denmark	635	44	679	0	0	0	6092	630	6722	4194	399	4593
Finland	314	0	314	15	0	15	3325	0	3325	2090	0	2090
France	4473	1254	5727	463	70	533	42755	14284	57039	27254	7642	34896
Germany	2919	282	3201	0	0	0	29544	2849	32393	19472	1839	21311
<b>Hungary</b>	<b>19</b>	<b>90</b>	<b>109</b>	<b>0</b>	<b>2</b>	<b>2</b>	<b>175</b>	<b>1195</b>	<b>1370</b>	<b>128</b>	<b>789</b>	<b>917</b>
Ireland	171	686	857	0	0	0	1868	7472	9340	1203	4810	6013
Italy	1900	75	1975	0	0	0	22000	695	22695	15000	487	15487
Luxembourg	160	20	180	0	0	0	0	0	0	1104	138	1242
Netherlands	1836	0	1836	0	0	0	16340	0	16340	11370	0	11370
Norway	35	25	60	0	0	0	0	0	0	203	158	361
Poland	151	0	151	41	0	41	1712	0	1712	1215	0	1215
Portugal	77	15	92	39	0	39	1413	186	1599	649	71	720
Russian Federation	142	708	850	448	0	448	6677	4803	11480	3495	4527	8022
Serbia	9	0	9	2	0	2	56	0	56	35	0	35
Slovenia	7	0	7	2	0	2	27	0	27	14	0	14
Spain	244	199	443	150	17	167	3729	2186	5915	2284	1340	3624
Sweden	192	7	199	5	0	5	1441	51	1492	967	27	994
Switzerland	323	24	347	137	1	138	4813	303	5116	3240	150	3390
Ukraine	2	0	2	0	0	0	7	0	7	6	0	6
United Kingdom	745	211	956	2	1	3	6297	1913	8210	3679	1107	4786
North America	17434	31288	48722	1161	25	1186	120981	368519	489500	111575	220677	332252
Canada	5838	2468	8306	1161	10	1171	6999	2478	9477	45947	18360	64307
Mexico	0	2096	2096	0	15	15	0	17943	17943	0	13289	13289



REGION/COUNTRY	BOVINE IN-VIVO DERIVED EMBRYOS															
	FRESH EMBRYOS			FROZEN DOMESTIC EMBRYOS				FROZEN IMPORTED EMBRYOS				EMBRYOS EXPORTED				
	DAIRY	BEEF	UNSORTED	DAIRY	BEEF	UNSORTED	TOTAL	DAIRY	BEEF	UNSORTED	TOTAL	DAIRY	BEEF	UNSORTED	TOTAL	
Africa																
South	1267	0	1382	35	638	0	673	453	735	0	1188	202	901	0	1103	
Zimba	12	0	12	0	0	0	0	0	0	0	0	0	0	0	0	
Africa Total	115	1279	0	1394	35	638	0	673	453	735	0	1188	202	901	0	1103
Asia																
Israel	161	0	0	161	16	0	0	16	0	10	0	10	0	0	0	0
Japan	2318	20324	0	22642	7288	44526	0	51814	1283	0	0	1283	0	0	0	0
Asia Total	2479	20324	0	22803	7304	44526	0	51830	1283	10	0	1293	0	0	0	0
Europe																
Austria	396	242	0	638	773	269	0	1042	40	12	0	52	35	21	0	56
Belgium	189	1006	0	1195	440	3030	0	3470	1426	34	0	1460	20	58	0	78
Denmark	2498	54	0	2552	966	124	0	1090	0	0	0	0	97	0	0	97
Estonia	0	0	0	0	0	0	0	0	0	6	6	12	0	0	0	0
Finland	892	0	0	892	1415	0	0	1415	252	0	0	252	67	0	0	67
France	15590	1729	48	17367	14321	6588	36	20945	1134	190	0	1324	1883	419	0	2302
Germany	7781	0	350	8131	11215	0	972	12187	0	0	0	0	0	0	0	0
Hungary	28	124	0	152	79	232	0	311	0	0	38	38	0	0	0	0
Ireland	573	2294	0	2867	629	2517	0	3146	0	0	0	0	0	0	0	0
Italy	6000	300	0	6300	0	0	0	0	0	0	0	0	0	0	0	0
Luxembourg	0	0	600	600	0	0	0	0	0	0	0	0	0	0	0	0
Netherlands	2522	0	0	2522	9519	0	0	9519	0	0	0	0	0	0	0	0
Norway	0	0	0	0	0	0	0	0	70	0	0	70	0	0	0	0
Poland	611	0	0	611	524	0	0	524	0	3	0	3	0	0	0	0
Portugal	120	10	0	130	434	9	0	443	4	63	0	67	0	0	0	0
Russian Federation	717	22	0	739	1290	4466	0	5756	82	143	0	225	0	0	0	0
Serbia	12	0	0	12	23	0	0	23	0	0	0	0	0	0	0	0
Slovenia	10	0	0	10	6	0	0	6	4	6	0	10	0	0	0	0
Spain	988	736	0	1724	752	408	0	1180	50	58	0	108	37	20	0	57
Sweden	0	0	78	78	0	0	744	744	0	0	181	181	0	0	0	0
Switzerland	859	21	0	880	1657	29	0	1686	374	16	0	390	48	0	0	48
Ukraine	0	0	0	0	0	0	0	0	35	0	0	35	0	0	0	0
United Kingdom	70	326	0	396	173	585	0	758	1	154	0	155	0	222	0	222
Europe Total	38556	6864	1076	4796	44216	18257	1752	64225	3472	685	225	4382	2187	740	0	2927
North America																
Canada	14762	2642	0	17404	18807	7821	0	26628	18	373	0	391	5135	3804	0	8939
Mexico	0	8031	0	8031	0	4022	0	4022	0	1486	0	1486	0	0	0	0
United States	29759	46290	0	76049	26457	108175	0	134632	0	0	0	0	7118	3640	0	10758
North America Total	44521	56963	0	101484	45264	120018	0	165282	18	1859	0	1877	12253	7444	0	19697

## Eredmények

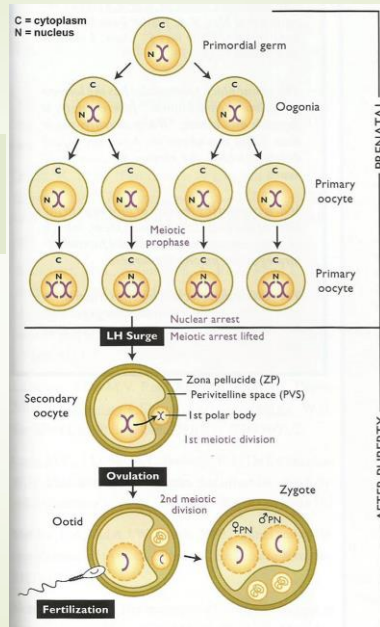
- HAHN J, HAHN R, BECZ J, PERJÉS I, SZILVÁSSY B: Megszületett az embrióátültetésből származó első borjú Magyarországon. Magy. Áo. Lapja, 1978 (3), 200.
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- SOLTI L.: A szarvasmarha embrió-átültetés. Magy. Áo. Lapja, 1981, 36, 186-189.
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- SOLTI L., CRICHTON E. G., LOSKUTOFF N. M., CSEH S.: Economical and ecological importance of indigenous livestock and the application of assisted reproduction to their preservation. Theriogenology 2000, 53 149-162
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## Eredmények

- 1988: Identikus borjak születnek (Brem és mtsai. 1988. Embriódarabolásból származó identikus szarvasmarha-ikerborjak. *Magy. Áo. Lapja*, 43.161.)
- 1991: első borjú IVF embrióból (Vajta és mtsai. 1991. Vemhesség in vitro fertilizált szarvasmarha petesejtéből. *Magy. Áo. Lapja*, 46.87.)
- 1992: magyarszürke borjú IVF embrióból (Bárándi és mtsai. 1992. In vitro fertilization as a new tool of preserving the Hungarian Grey Cattle. In: Alderson, L. and Bodó, I. eds.: *Genetic Conservation of Domestic Livestock Vol. II*, 271-274, CAB International, Wallingford, UK.
- 1994: első borjú mélyhűtött IVF embrióból (Enying) (Seregi és mtsai. 1995. The first successful transfer of frozen embryos derived from IVMFC of bovine oocytes in Hungary. *Theriogenology*, 43.190.)
- 1995: első báránycsib IVF embrióból (Cseh és mtsai. 1995. In vitro fertilizációval előállított juh embriók sikeres átültetése. *Magy. Áo. Lapja*, 50.829., Treuer és mtsai. 1996. Successful implantation of goat and IVMFC ovine embryos by laparoscopy technique. *J. of Physiology and Pharmacology*, 47.163.)
- 1996: megszületik az első szarvas utód ET-ből (Zomborszky és mtsai. 1998. Sperm collection from shot red deer stags (*Cervus Elaphus*) and the utilisation of sperm frozen and subsequently thawed. *Acta Veterinaria Hungarica*, 47.263.)
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## Az oogenesis főbb lépései

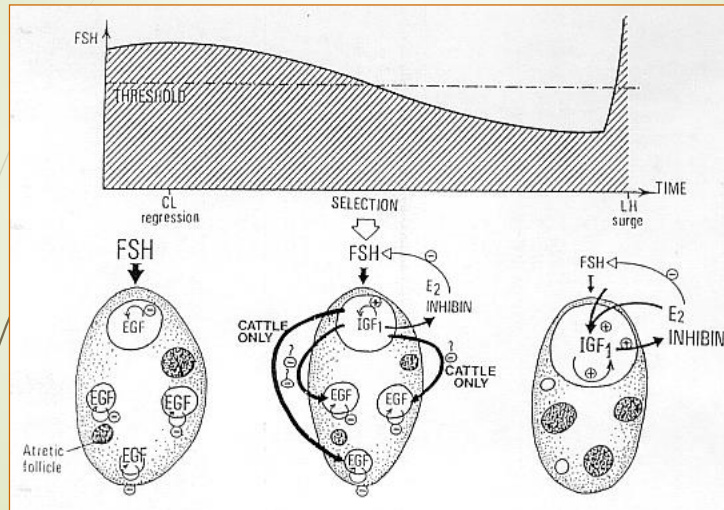


**Prenatal**  
Oogenesis begins with the development of primordial germ cells in the embryo. Primordial germ cells divided mitotically into oogonia. Oogonia divided into primary oocytes that enter the first meiotic prophase. At the end of meiotic prophase the nuclear material is arrested. This arrest is called the dictyate, a form of nuclear "hibernation".

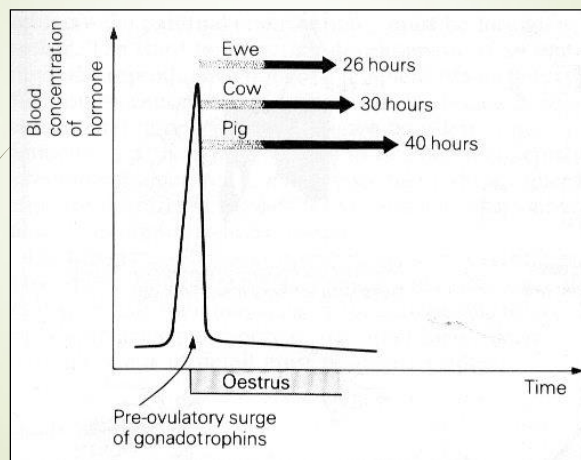
**After Puberty**  
At puberty, the female begins to cycle and ovulate. The LH surge allows the meiotic arrest to be lifted in the first meiotic division takes place. This division results in the formation of a secondary oocyte that possesses the first polar body. The first polar body contains one-half of the genetic material. Around the time of ovulation, the second polar body is voided and the ootid is formed. Fertilization occurs slightly before or slightly after the second meiotic division. At fertilization the sperm delivers the other half of the genetic material and a zygote is formed. At this time the zygote contains a male and a female pronucleus. When the pronuclei fuse, early embryo development begins.



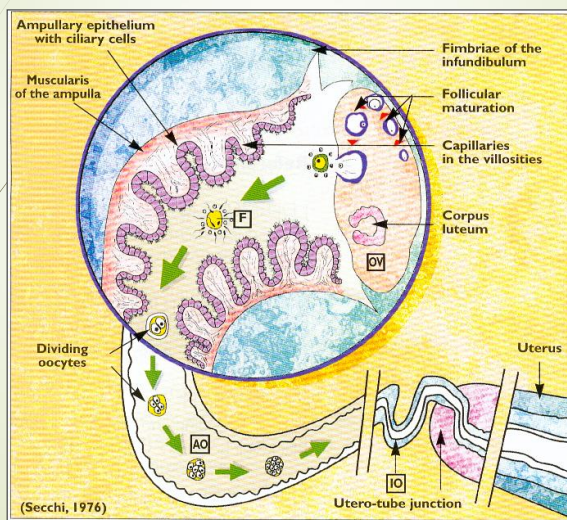
## Tüszőnövekedés 2



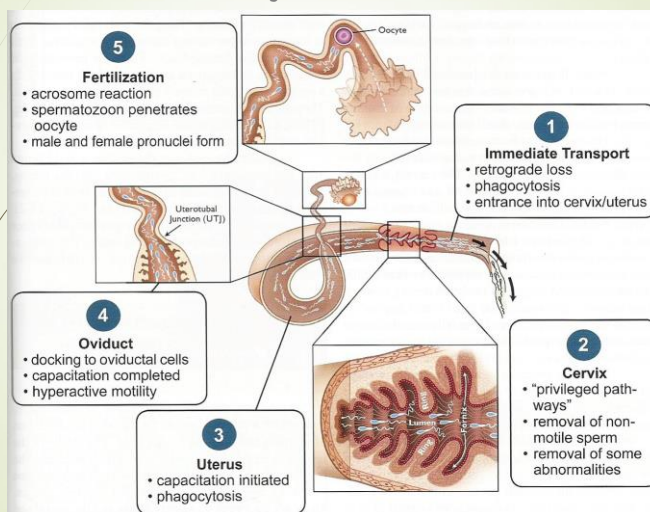
## LH csúcs → ovuláció



## Oviductus transzport - fertilizáció



## Események a sperma bejutása után





## Kapacitáció és acrosoma reakció

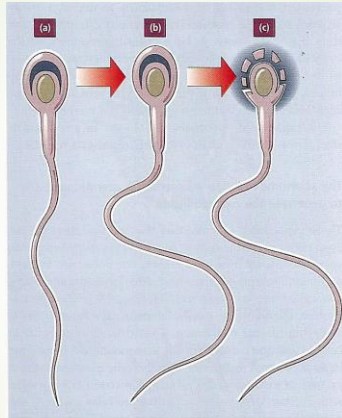
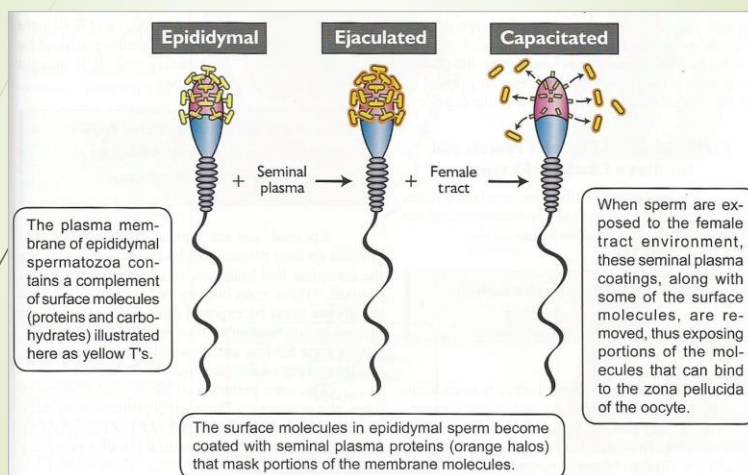
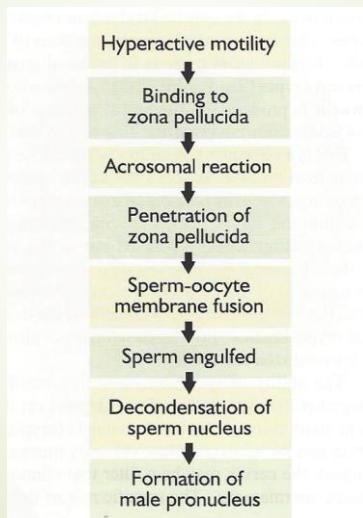


Fig. 9.4 (a) Schematized spermatozoon prior to capacitation; a consequence of capacitation is (b) hyperactivated tail movements, and development of the capacity subsequently to undergo (c) the acrosome reaction, in which multiple sites of fusion between the plasma membrane and the outer acrosomal membrane 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.

## Kapacitáció

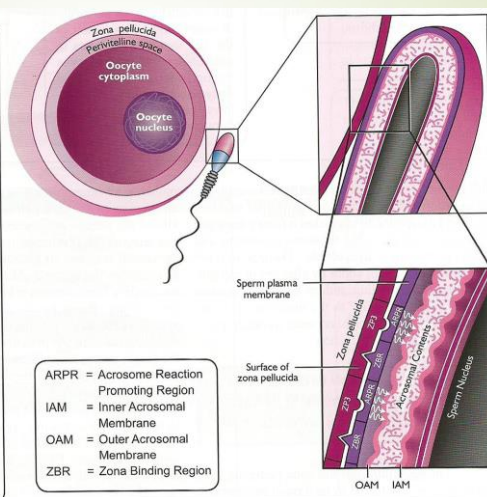


## Kapacitáció utáni események, fertilizáció

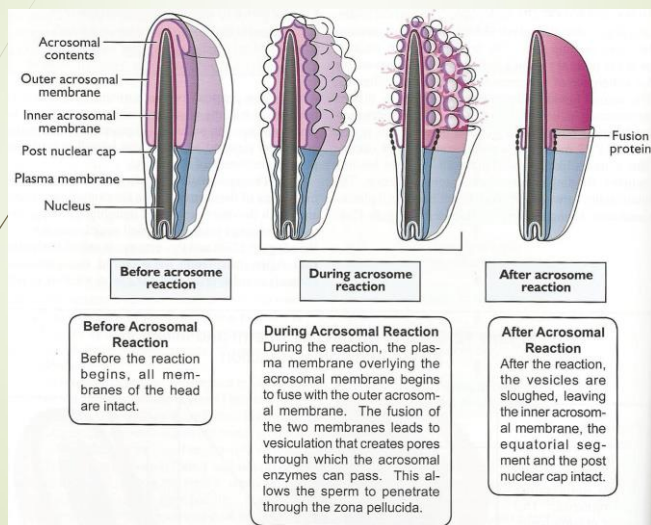


## Zona binding, az acrosoma reakció kezdete

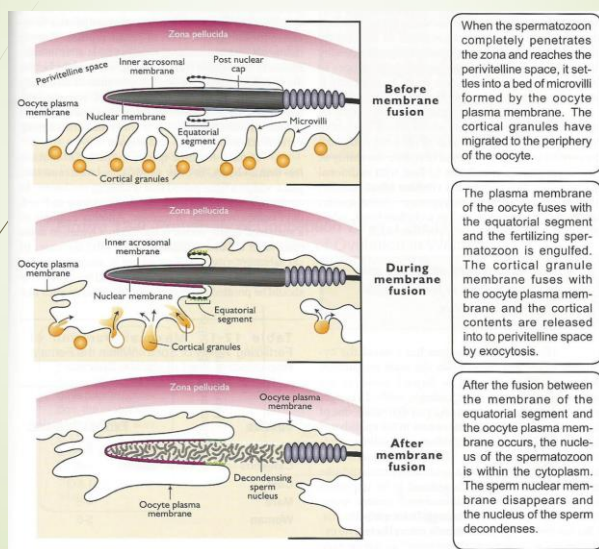
Proposed model for zona binding and the initiation of the acrosomal reaction in mammalian spermatozoa. The sperm plasma membrane overlying the acrosome contains two receptor-like regions. The first, called the zona binding region (ZBR), reacts with ZP3 to cause physical attachment of the sperm to the zona pellucida. A second membrane region, the acrosome reaction promoting region (ARPR), also binds to ZP3 and initiates the acrosome reaction by causing the sperm plasma membrane to fuse (arrows) to the outer acrosomal membrane.



## Acrosoma reakció



## Spermium-oocyta fúzió





## Ca<sup>2+</sup> hullámok a petesejtben

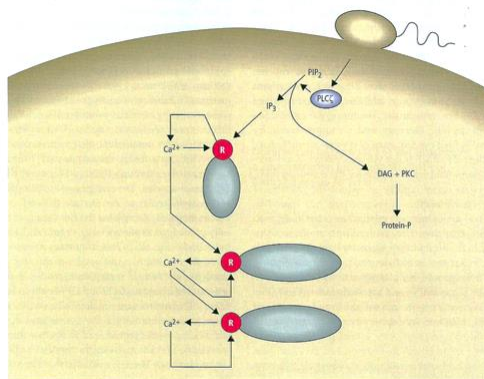


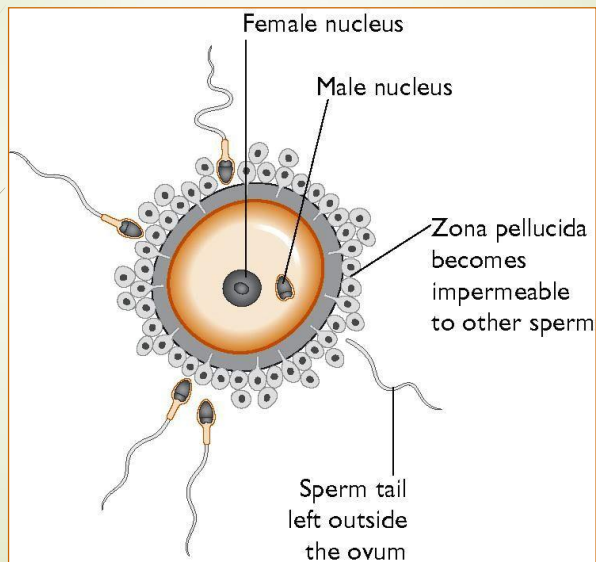
Fig. 9.7 Model for activation of the oocyte calcium waves by the fertilizing spermatozoon. After fusion, the spermatozoon introduces phospholipase C $\zeta$  (*zceta*) into the oocyte. The PLC $\zeta$  stimulates the release of the second messengers *inositol triphosphate* (IP $_3$ ) and *diacylglycerol* (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.

## Embriófejlődés, események az anyában

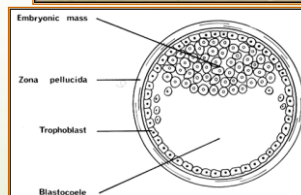
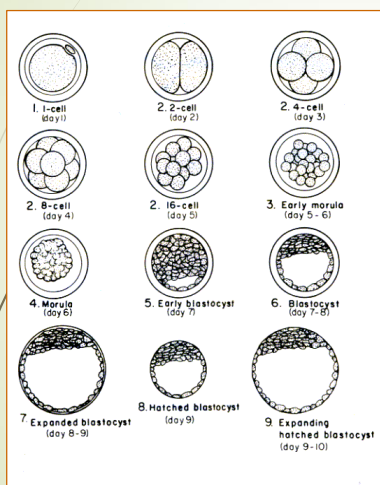
Table 10.1 Times (in days) after ovulation at which various developmental and maternal events occur.

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
<i>Invasive</i>							
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
<i>Non-invasive</i>							
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

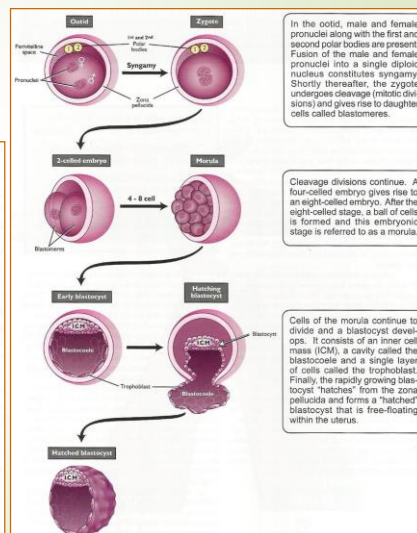
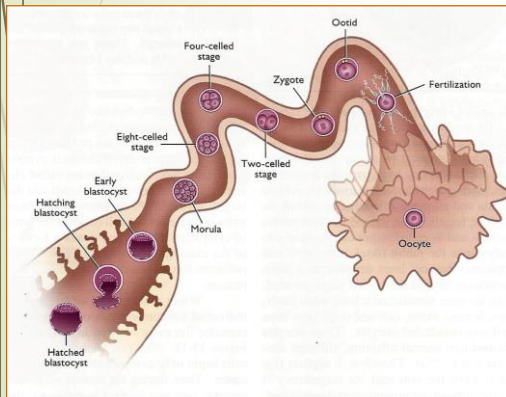
## Oviductus transzport - fertilizáció



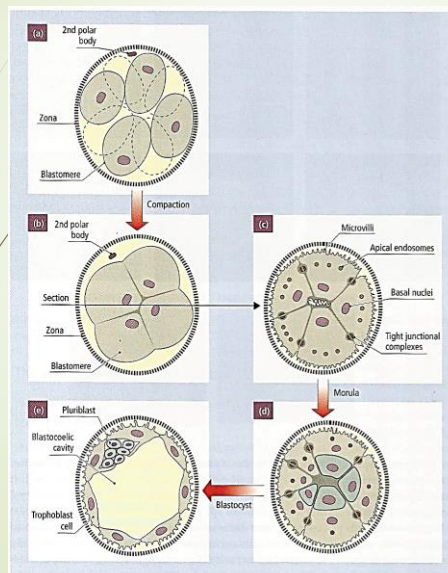
## Korai embriófejlődés



# Oviductus transport



# Kompaktálódás és polarizáció



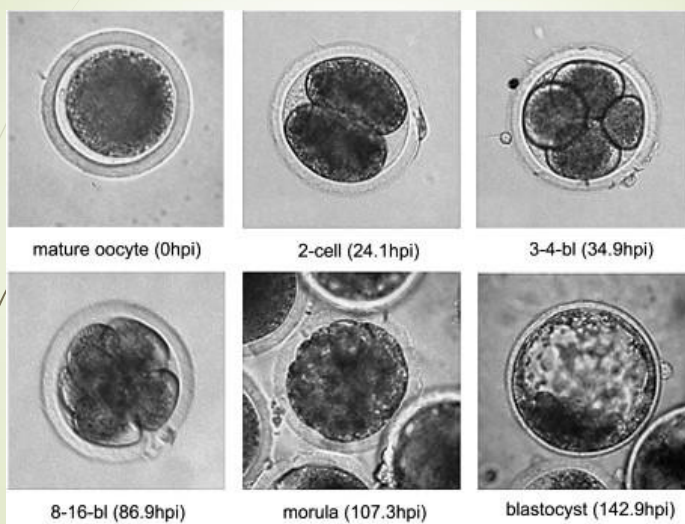
**Fig. 10.2 (a-c)** Compaction of eight-cell conceptus. Spherical cells (a) become wedge-shaped (b,c) and, by apposing adjacent surfaces, maximize cell contact. In cross-section (c), it can be seen that tight junctional complexes develop between the outer membranes of adjacent cells; these are punctate 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.



## Korai embriófejlődés

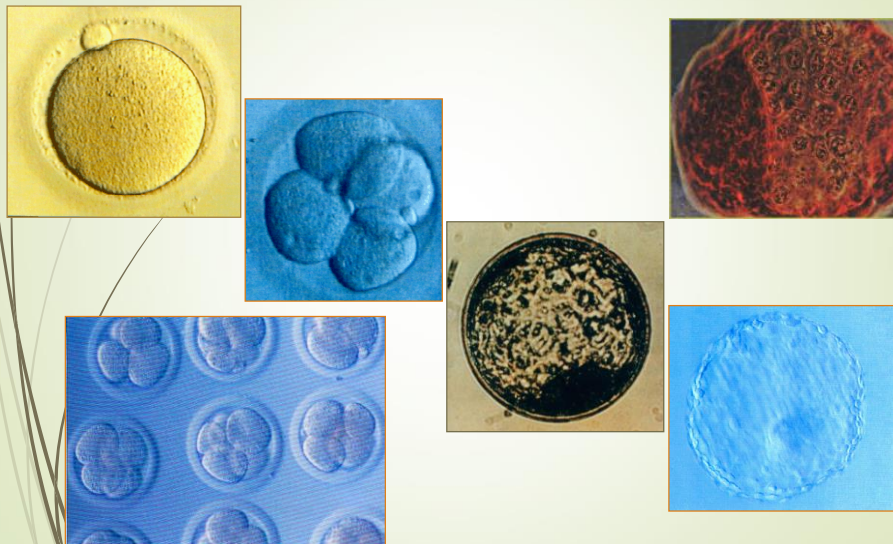


## Korai embriófejlődés

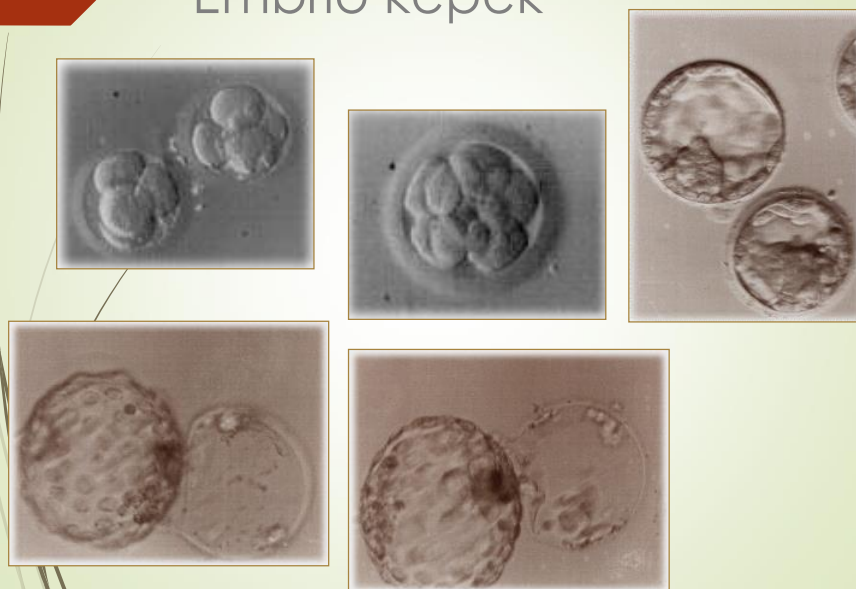




## Korai embriófejlődés

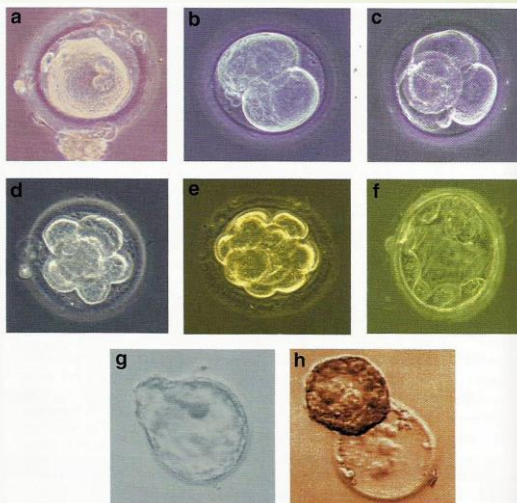


## Embrió képek

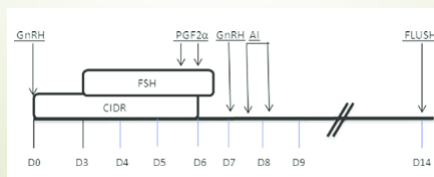
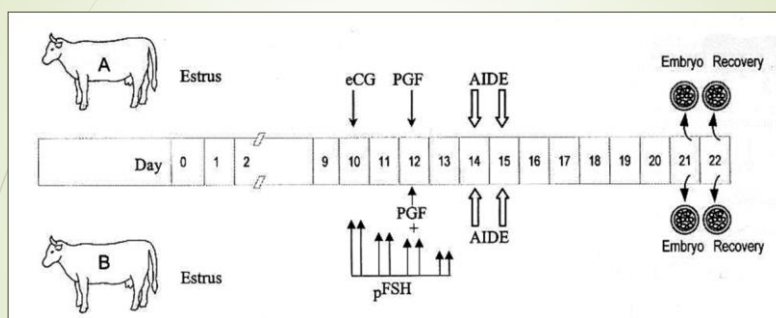


## Humán embriók fejlődése

**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.)



## Szuperovulációs protokollok

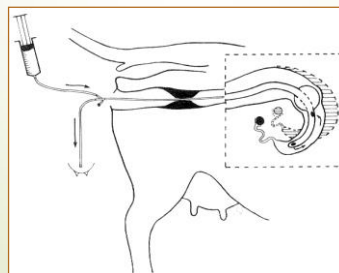
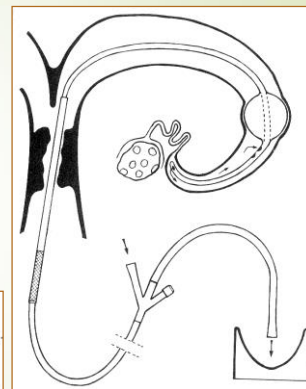
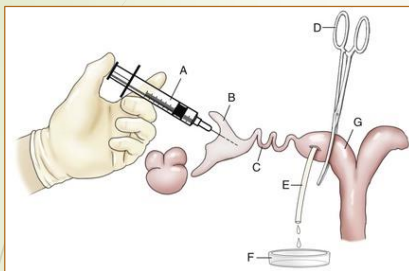




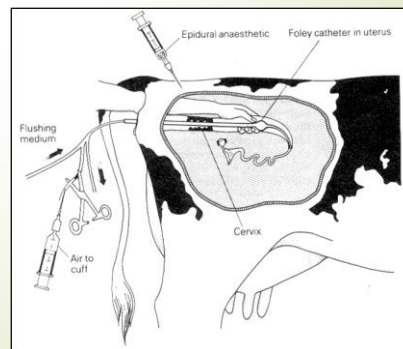
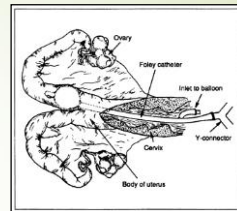
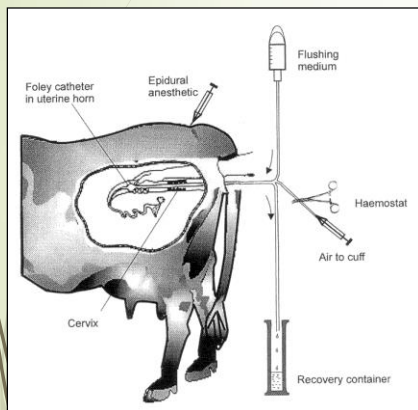
## Szuperovulációs válaszreakció



## Embriónyérés



## Nem sebészi embriónyerés



## A mosófolyadék

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 <sub>2</sub> atmosphere)*
Sterilization	Filtration of medium through 0.22- $\mu$ m-pore membranes, aseptic techniques; sterile equipment; addition of 100 IU penicillin G, and 50 $\mu$ g streptomycin sulphate per ml, or 25 $\mu$ g/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

## Dulbecco PBS (10 l-hez)

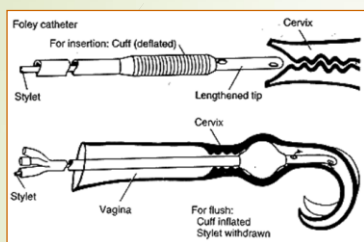
Mixture One	Amount	Function
CaCl <sub>2</sub> ·2H <sub>2</sub> O	1.32 g	Membrane/enzyme function
MgSO <sub>4</sub> ·7H <sub>2</sub>	1.21 g	Membrane/enzyme function
The above may be weighted in advance and stored indefinitely in a sterile bottle under refrigeration		
Mixture Two	Amount	Function
NaCl	80.0 g	Osmotic balance; neutralize charge cell membrane
KCl	2.0 g	
Na <sub>2</sub> HPO <sub>4</sub>	11.5 g	Buffer to maintain pH
KH <sub>2</sub> PO <sub>4</sub>	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 microorganisms

Mixture Two may be weighed in advance and stored dry in a sterile bottle under refrigeration for six months

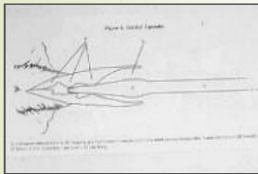
### Combination of mixtures One and Two

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 katéter (Rüsch)



## Cervix tágító, mosófolyadék



Embrió  
kimosás



## Embrió kimosás



## Embriók izolálása



# Embrió szűrők

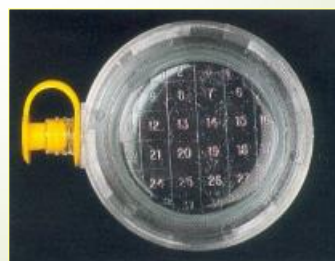
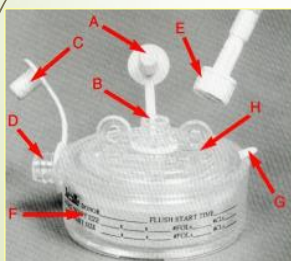
**MINIFLUSH™ EMBRYO RECOVERY SYSTEM**

**ADVANTAGE OVER ADVANTAGE**

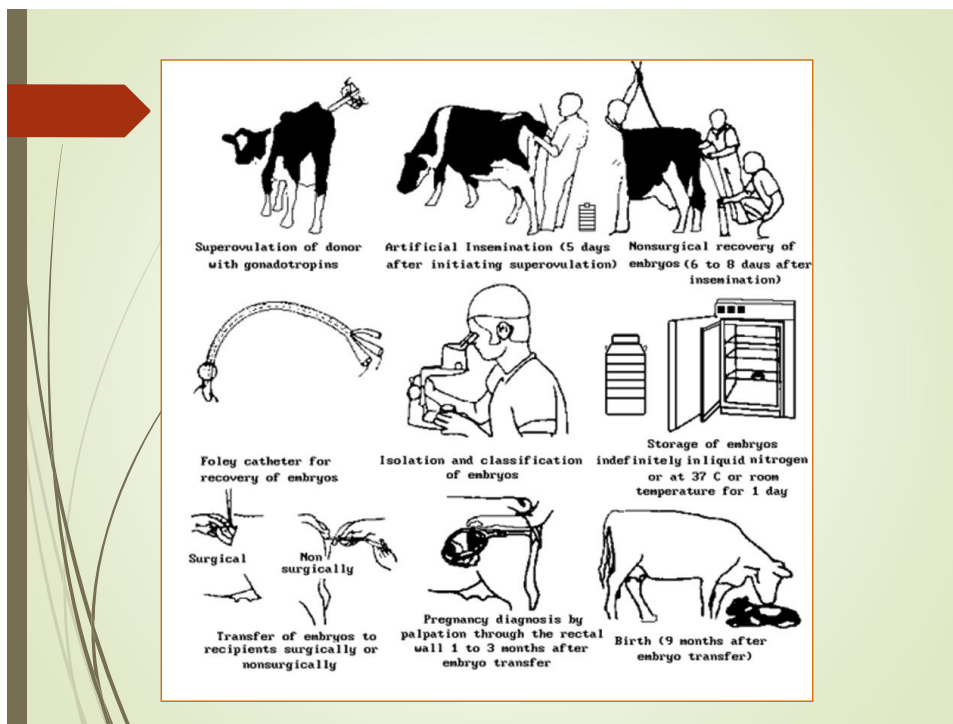
- Small footprint system. Fits inside 2500
- Enhanced filter advantages (lighter)
- Filter area is 4x larger than standard
- New type filters
- Synthetic Filter Area
- Automatically Inflates. Maximum Flow (10ml)
- Suitable for Current Practice Treatment and
- Culture Media
- Filter is replace (or it better) during
- Multiple Procedures
- Embryos are recovered directly from Re-
- covery (RISQ) tube
- Made in the U.S.A.



# Embrió szűrők







## Embrióbírálat: fejlődési stádium

(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.



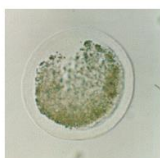
# Embrióbírálat: Minőség

(Bó and Mapletoft, 2013)

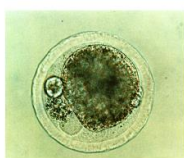
- **Code 1: Excellent or Good.** The embryos have a symmetrical and spherical mass with individual blastomeres that are uniform in size, color, and density. This embryo is consistent with its expected stage of development. Irregularities should be relatively minor, and at least 85% of the cellular material 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.

## Szmha embriók: fejlettség és minőség

### Fejlettség: 1-5



Cycle Day: 7  
Stage Code: 1  
Quality Code: 4



Cycle Day: 7  
Stage Code: 1  
Quality Code: 4



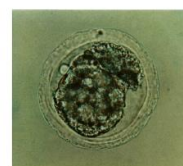
Cycle Day: 7  
Stage Code: 1  
Quality Code: 4



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



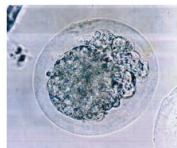
Cycle Day: 7  
Stage Code: 4  
Quality Code: 1



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

## Szmha embriók: fejlettség és minőség

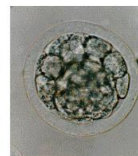
### Fejlettség: 1-5



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



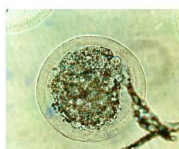
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Stage Code: 4  
Quality Code: 3



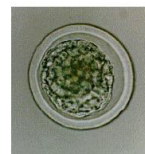
Cycle Day: 7  
Stage Code: 4  
Quality Code: 3



Cycle Day: 7  
Stage Code: 4  
Quality Code: 3



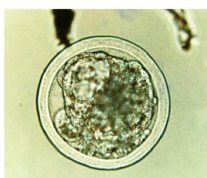
Cycle Day: 7  
Stage Code: 4  
Quality Code: 3



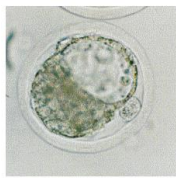
Cycle Day: 7  
Stage Code: 5  
Quality Code: 1

## Szmha embriók: fejlettség és minőség

### Fejlettség: 5-9



Cycle Day: 7  
Stage Code: 5  
Quality Code: 2



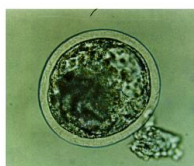
Cycle Day: 7  
Stage Code: 5  
Quality Code: 1



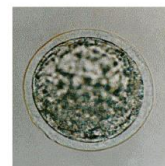
Cycle Day: 7  
Stage Code: 5  
Quality Code: 2



Cycle Day: 7.5  
Stage Code: 5  
Quality Code: 1



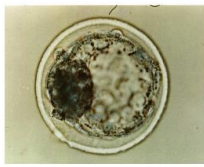
Cycle Day: 7.5  
Stage Code: 6  
Quality Code: 1



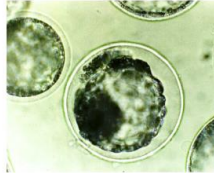
Cycle Day: 7.5  
Stage Code: 6  
Quality Code: 1

## Szmha embriók: fejlettség és minőség

### Fejlettség: 5-9



Cycle Day: 7,5  
Stage Code: 7  
Quality Code: 1



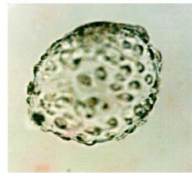
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Stage Code: 7  
Quality Code: 2



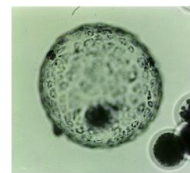
Cycle Day: 7,5  
Stage Code: 7  
Quality Code: 2



Cycle Day: 8  
Stage Code: 8  
Quality Code: 1

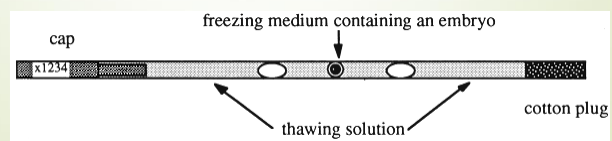
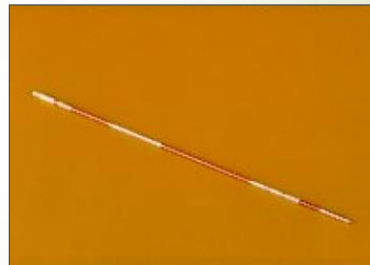


Cycle Day: 8  
Stage Code: 8  
Quality Code: 1

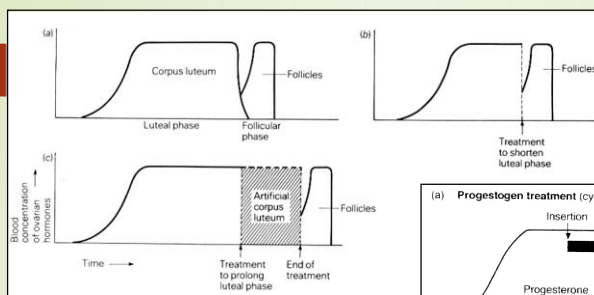
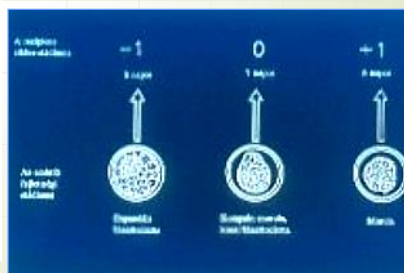
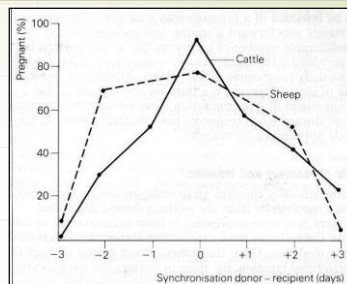
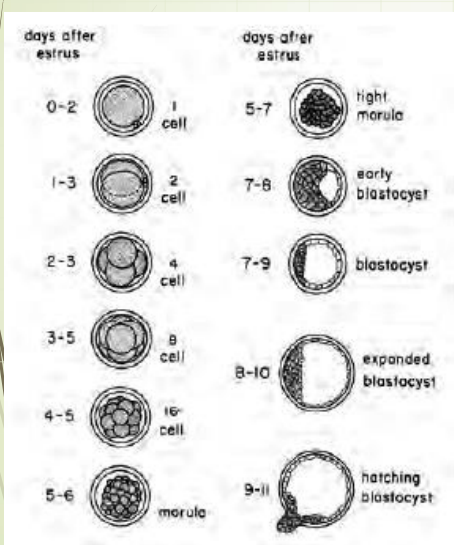


Cycle Day: 9  
Stage Code: 9  
Quality Code: 1

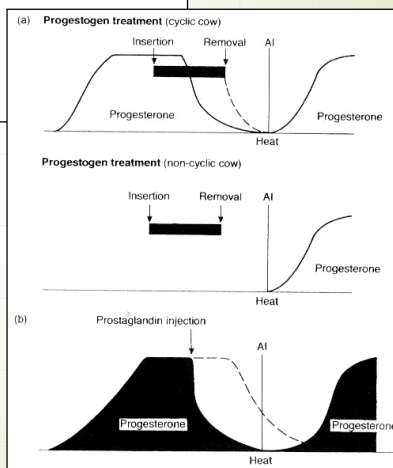
## Az embriók betöltése



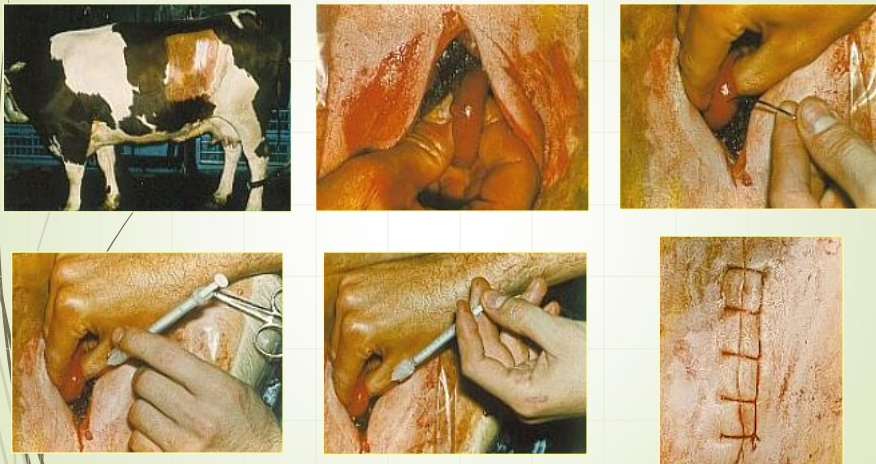
# Donor-recipients szinkronizálás



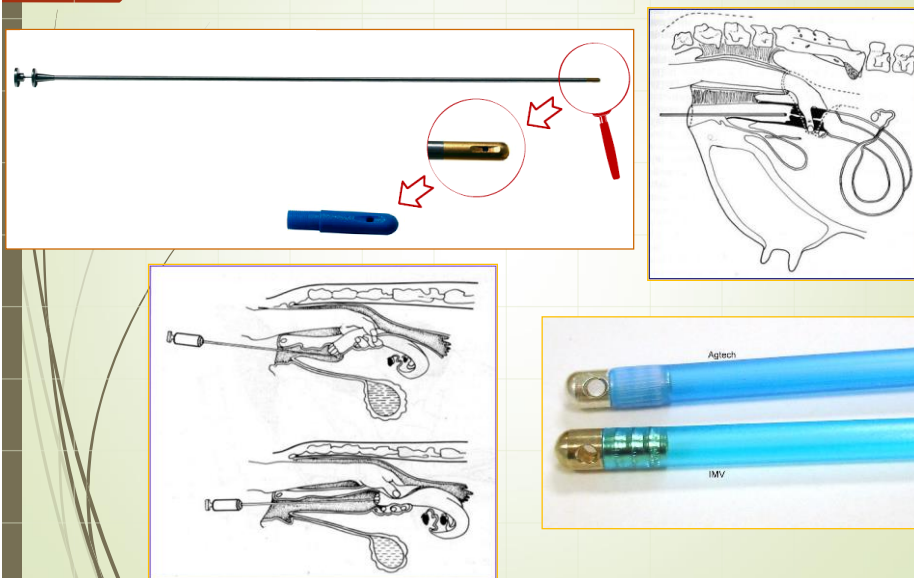
## Donor-recipients szinkronizálás



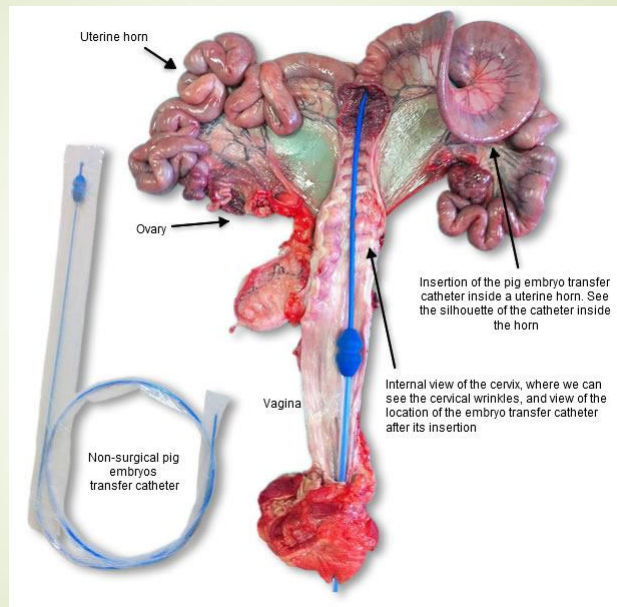
## Sebészi embrióbeültetés



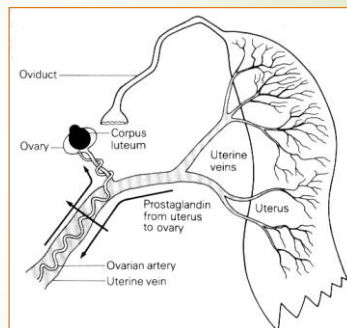
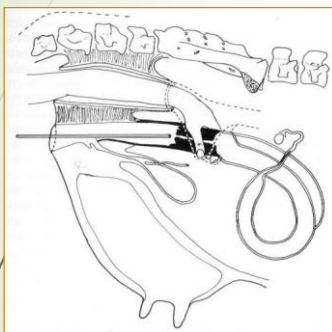
## Beültető katéter







## Ipsilateralis beültetés



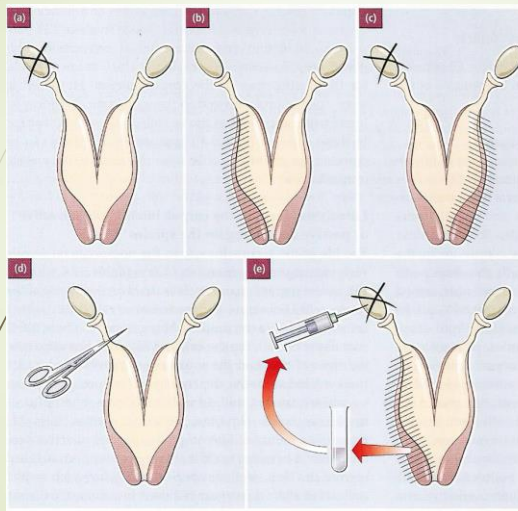


Fig. 5.6 Non-pregnant sheep uterus and ovaries. (a) A single corpus luteum present in the left ovary regresses, indicated by a cross. (b) Removal of the ipsilateral uterine horn (hatched) prevents regression. (c) Removal of the contralateral horn does not prevent regression. (d) Clamping the blood supply 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).

## Várható eredményesség

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-Trägartiere (Anteil gegenüber betriebseigenen Trägern)	237	255
Trächtigkeitsrate (%)	51,1 %	54,7 %
Anzahl tiefgefrorener Embryonen	658	903
Trächtigkeitsrate bei OHG-Trägartieren 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)



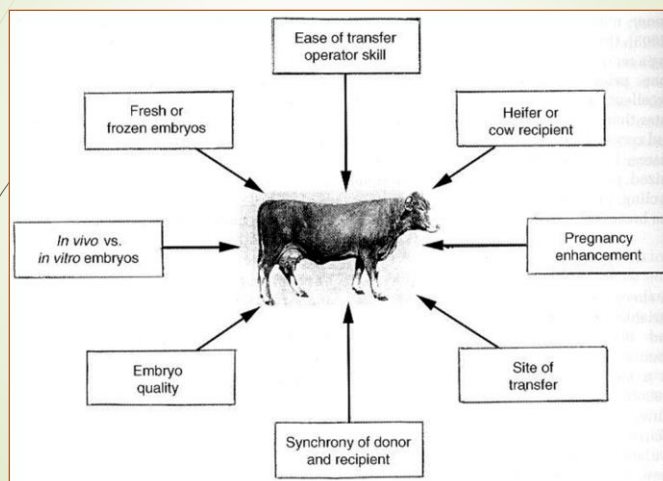
## Várható eredményesség

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

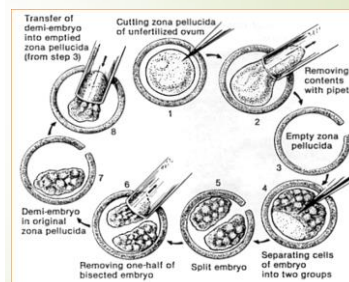
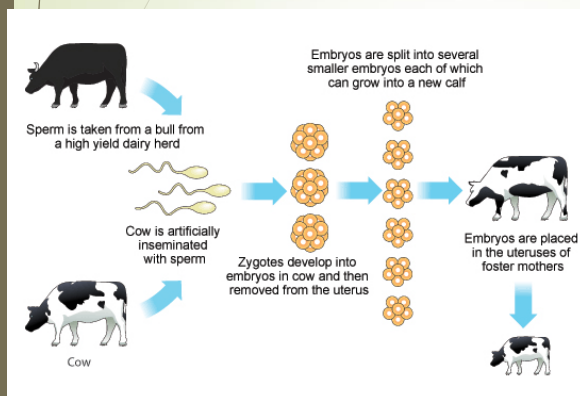
Spendertier: Vater	Besitzer	gefundene Embryonen	transfertaugliche Embryonen	Anpaarungs- bulle
<b>Molly DT</b> Dorado	Niederwestberg, Oberschlochtern	40	30	Virzil
<b>Wonder Red</b> Jordan Red	Gülker, Haldem	26	26	Lawn Boy
<b>Beka FT</b> Lancelot	Pues-Tillkamp, Glandorf	31	21	Bertil
<b>Saint DT</b> Convincer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

(Source: Osnabrücker Schwarzbuntzucht 2009/1)

## A sikerességet befolyásoló tényezők

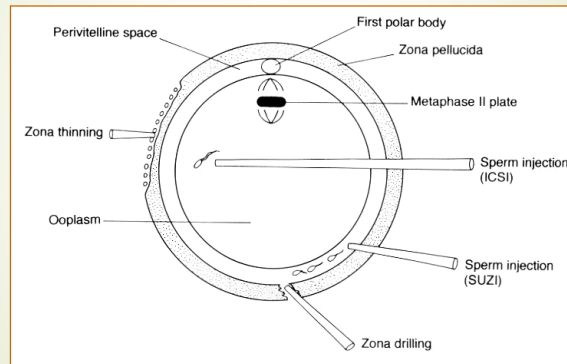


## Az embriók darabolása

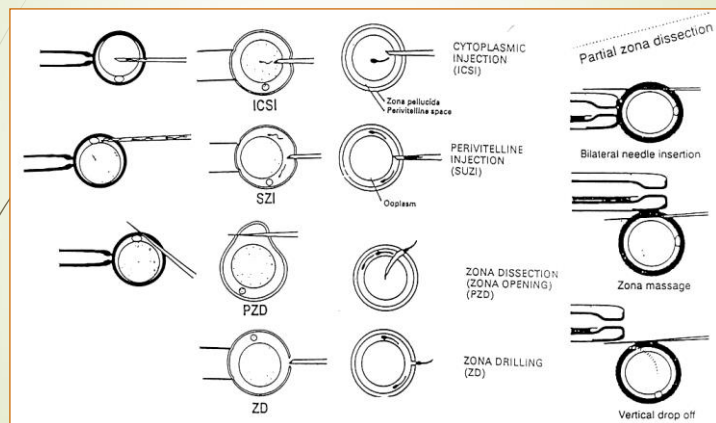


## Asszisztált reprodukciós eljárások (Kiegészítő módszerek)

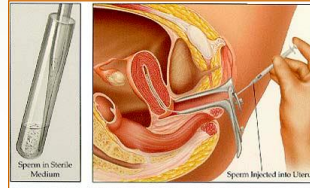
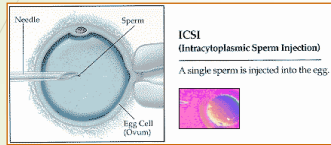
## Asszisztált reprodukciós technikák



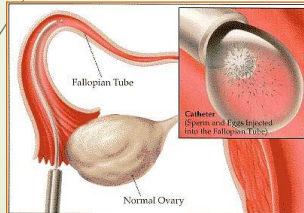
## Asszisztált reprodukciós technikák



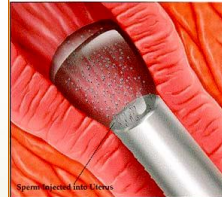
# ART



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.

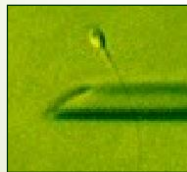
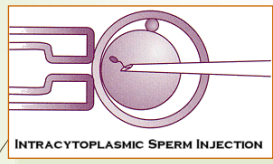


After the retrieval of the eggs from the ovary, both sperm and eggs are injected through the catheter directly into the fallopian tube. Fertilization may then take place normally in the fallopian tube.



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

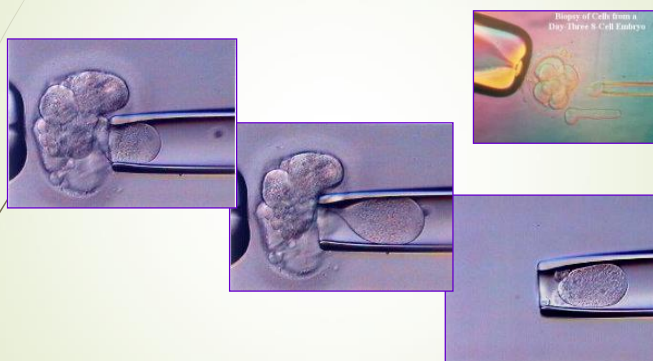
# Mikrofertilizáció (ICSI, SUZI)



## Asszisztált hatching

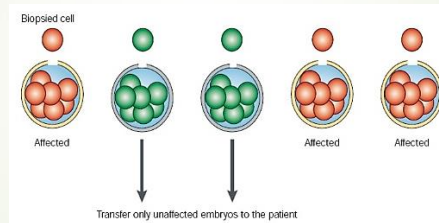


## Embrió biopszia - prenatalis diagnosztika



## 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



Family Balancing

