HODNOCENÍ OOCYTŮ A EMBRYÍ

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Proč je nutné provádět honocení?

Získáváme více než jedno:

- **vajíčko**
- **zygotu**
- **embryo**

Transferujeme 2 až 3 embrya.

Co hodnotíme a kdy?

- oocyty po OPU – oocyt - cumulární –komplet
- denudované oocyty před ICSI
- zygoty po fertilizaci
- jednotlivé vývojové stadia embryí od 2bb až po blastocystu

Požadavky na hodnocení

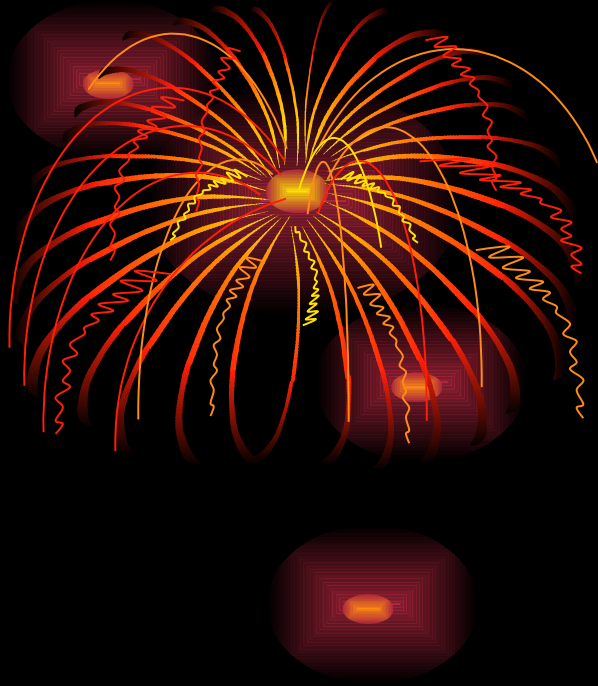
- rychlé
- efektivné
- neinvazivné

Hodnocení COC

Je nutné hodnotit oocyty po OPU?

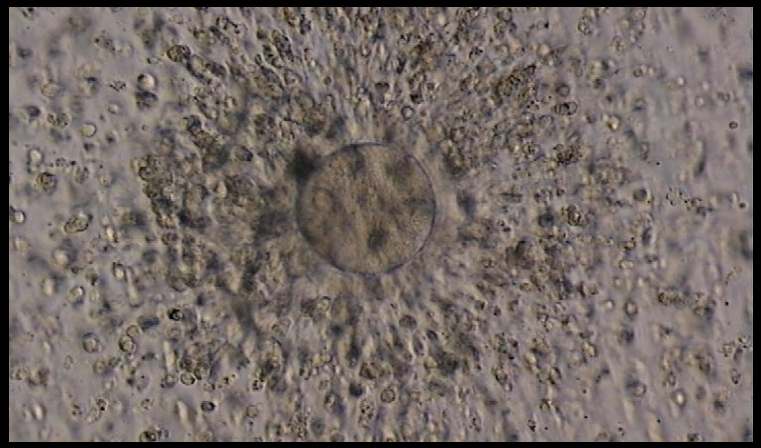
- **možnost dokultivovat nezralé oocyty a inseminovat je až v době jejich zralosti**
- **předpoklad výsledku inseminace???**

Hodnocení COC



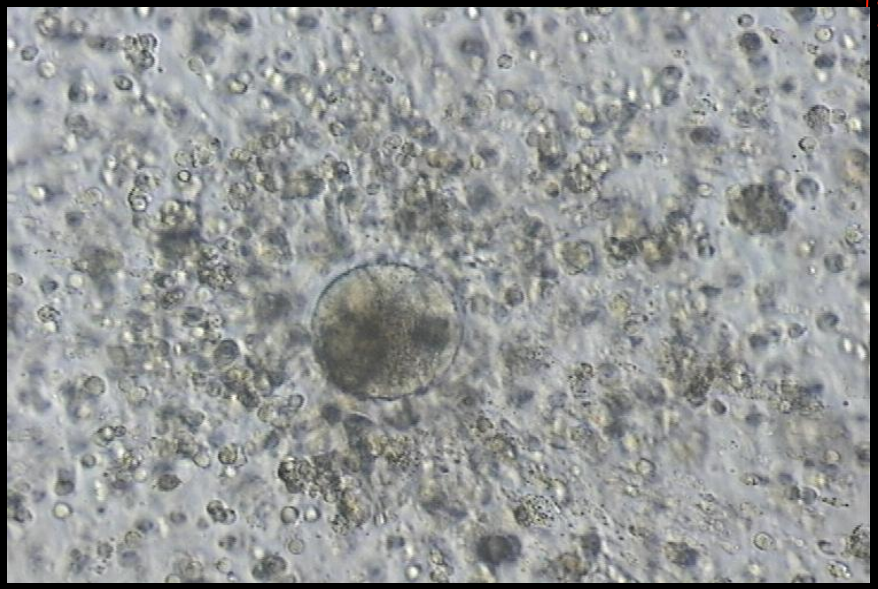
- Zralý
- částečně zralý
- nezralý

Zralý oocyt – mature oocyt



Kumulus - plně expandovaný
Korona radiata – buňky jsou bledé,
nevýrazné a medové barvy

Částečně zralý oocyt
intermediate oocyt



Kumulus – částečně expandovaný.
Korona radiata – částečně expandovaná.

NEZRALÝ OOCYT – imature oocyte

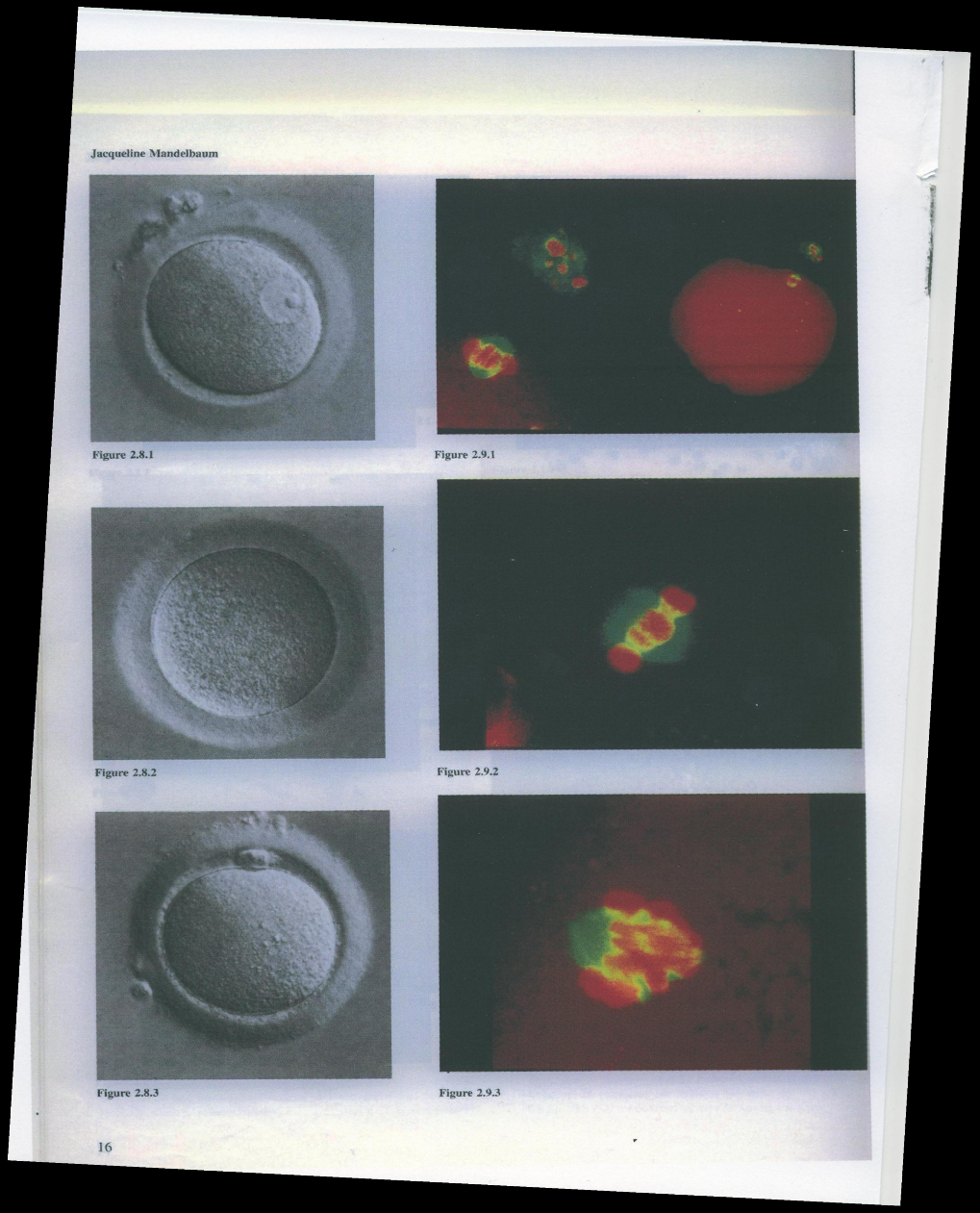
Kumulus – hustý, buňky neexpandované.

Korona radiata – hustá, tmavá. Tmavá ooplazma.

Denudované oocyty

Hodnotíme:

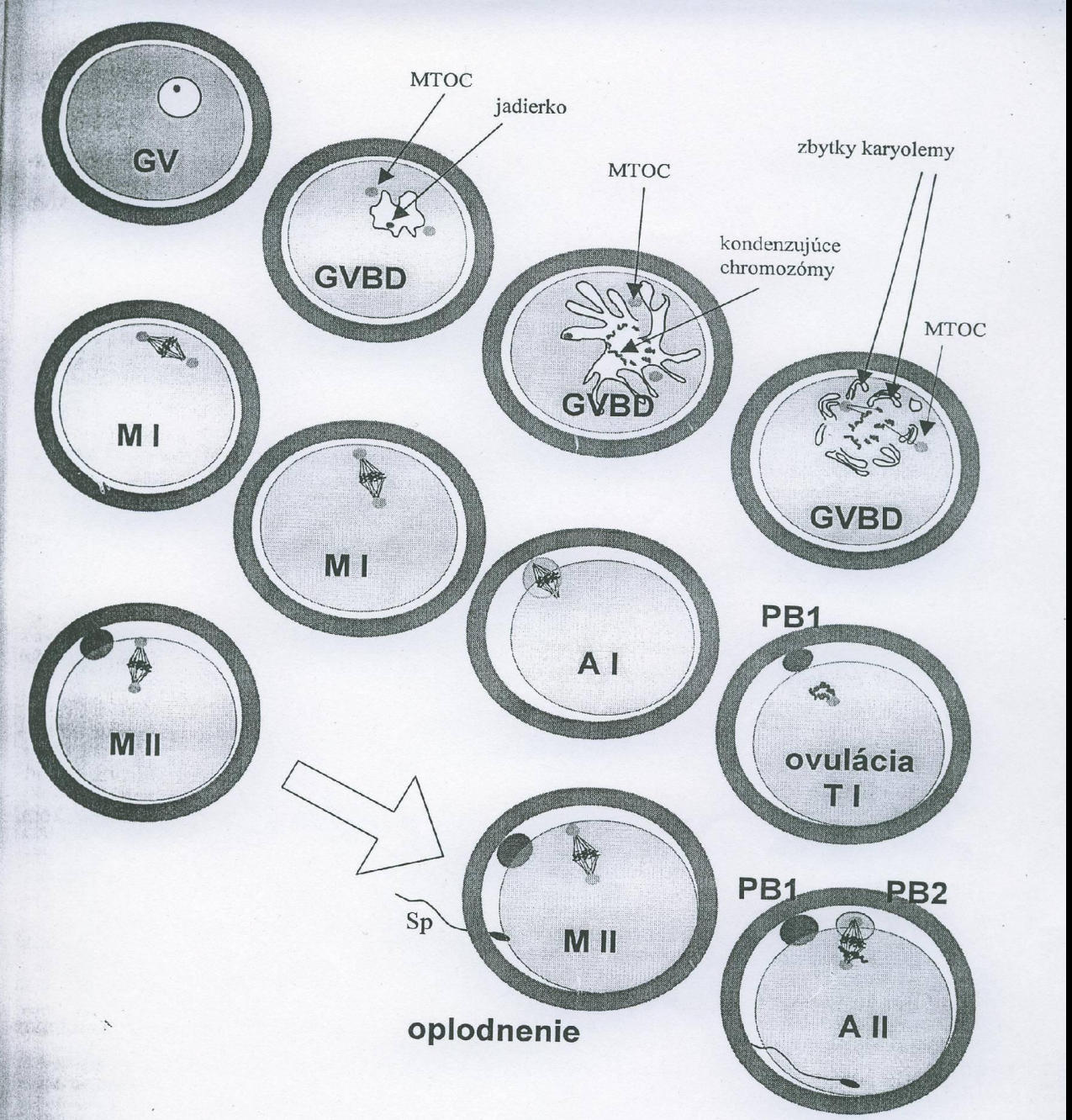
- **výskyt nebo nepřítomnost zárodečného váčku**
- **přítomnost, nepřítomnost polového tělíska a jeho tvar (fragmentace)**
- **zralost cytoplazmy**
- **zralost jádra**



GV – nezralý oocyt dozrává 26 až 29 hod po OPU

M1 – nezralý oocyt inseminace 1 až 5 hod po extruzi 1. PT

M2 - Zralý oocyt inseminace 2 až 5 hod po OPU



Obrázok 4. Schéma dozrievania oocytu od štádia zárodočného mechúrika po druhé meiotické delenie.

Schéma procesu zrání vajíčka od GV stádia až po inseminaci

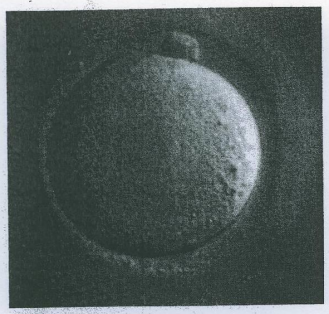


Figure 3.13.1

Figure 3.13.2

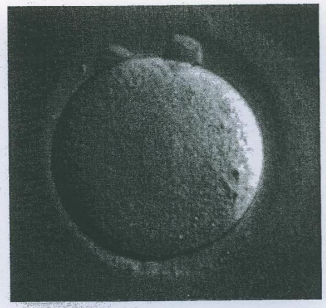


Figure 3.13.3

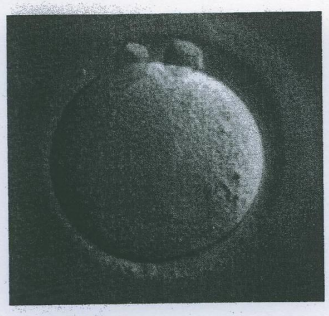


Figure 3.13.4

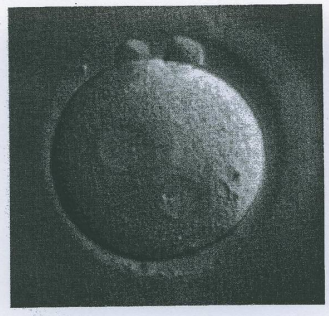


Figure 3.13.5

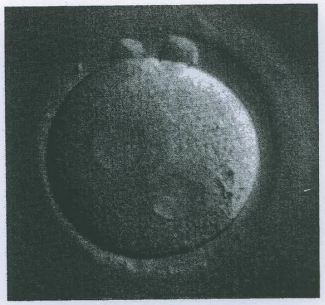


Figure 3.13.6

Figure 3.13.7

Figure 3.13.8

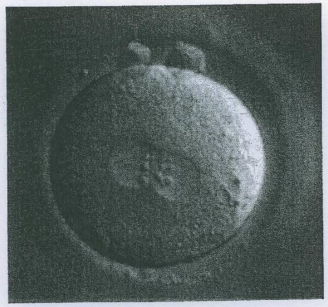


Figure 3.13.9

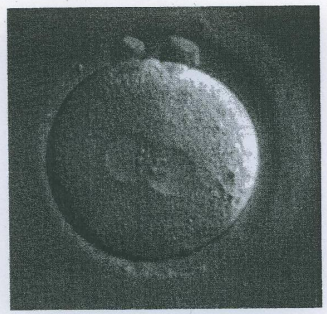


Figure 3.13.10

Vývoj po inseminaci

Detail extruze polového tělíska

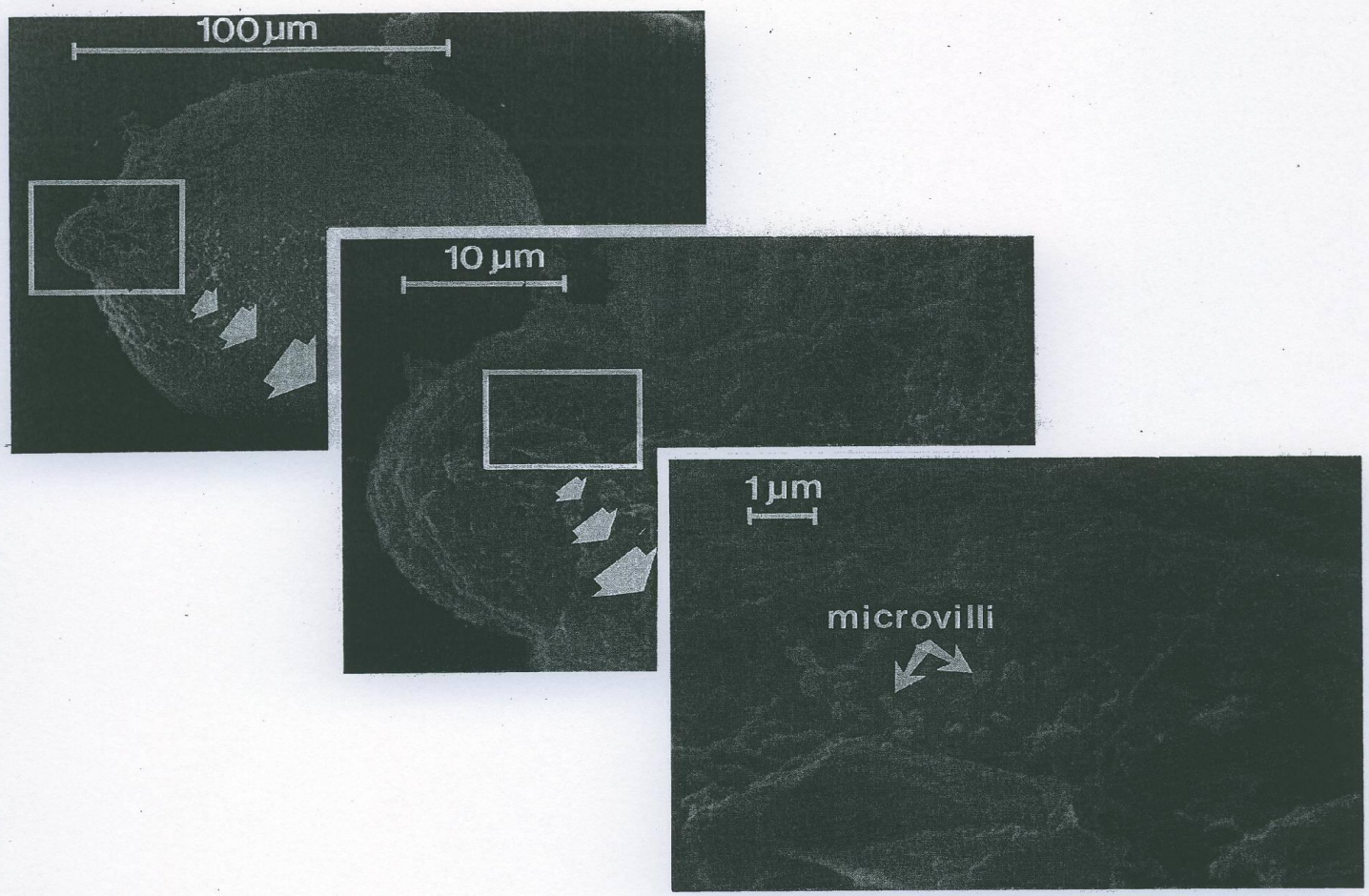


Figure 6.30

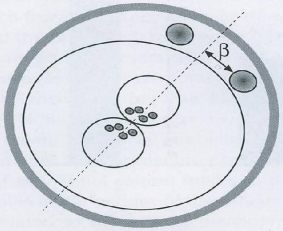
at the pronuclear stage in those transferred led to a pregnancy rate of 22/44 (50%) compared with only 2/23 (9%) when none were present.

A further criterion of pronuclear embryos that may effect embryo morphology is the orientation of pronuclei relative to the polar bodies. Oocyte polarity is clearly evident in non-mammalian species. In mammals, the animal pole of the oocyte may be estimated by the location of the first polar body, whereas after fertilization, the second polar body marks the embryonic pole.[18] In human oocytes a differential distribution of various factors within the oocyte has been described and anomalies in the distribution of these factors, in particular the side of the oocyte believed to contain the animal pole, are thought to affect embryo development and possibly fetal growth.[19,20] Following from this hypothesis Garello et al[21] examined pronuclear orientation, polar body placement, and embryo quality to ascertain if a link existed between a plausible polarity of oocytes at the pronuclear stage and further development. The most interesting observation involved the calculation of angle β (Fig 17.1), which represented the angle between a line drawn through the axis of the pronuclei and the position of the furthest polar body. They found that as the angle β increased there was a concurrent decrease in the morphological quality of preimplantation stage human embryos. They postulated that the misalignment of the polar body might be linked to cytoplasmic turbulence hence disturbing the delicate polarity of the zygote. A further study by Scott and Smith[22] devised an embryo score on day 1 on the basis of alignment of pronuclei and nucleoli, the appearance of the cytoplasm, nuclear membrane breakdown and cleavage to the 2 cell stage. Patients who had an overall high embryo score (≥15) had a pregnancy and implantation rate of 34/48 (71%) and 49/175 (28%) respectively, compared with only 4/49 (8%) and 4/178 (2%) in the low embryo score group.

CLEAVAGE STAGE EMBRYOS

The most widely used criteria for selecting the best embryos for transfer have been based on cell number and morphology.[7] A vast number of variations on the theme have been published, however, some recent studies by Gerris et al[23] and Van Royen et al[5] used strict embryo criteria to select single embryos for transfer. The necessary characteristics of their "top" quality embryos were established by retrospectively examining embryos that had a very high implantation potential.[5] These "top" quality embryos had the following characteristics: four or five blastomeres on day 2 and at least seven blastomeres on day 3 after fertilization, absence of multinucleated blastomeres and <20% of fragments on day 2 and day 3 after fertilization. When these criteria were used in a prospective randomized clinical trial comparing single and double embryo transfers it was found that in 26 single embryo transfers, where a top quality embryo was available, an implantation rate of 42.3% and ongoing pregnancy rate of 38.5% was obtained. In 27 double embryo transfers an implantation rate of 48.1% and ongoing pregnancy rate of 74% was obtained.

Most studies that have used and report embryo selection criteria on the basis of cell number and morphology do so by stating that embryos were selected on day 2 or day 3. As discussed by Bavister[16] one of the most critical factors in determining selection criteria



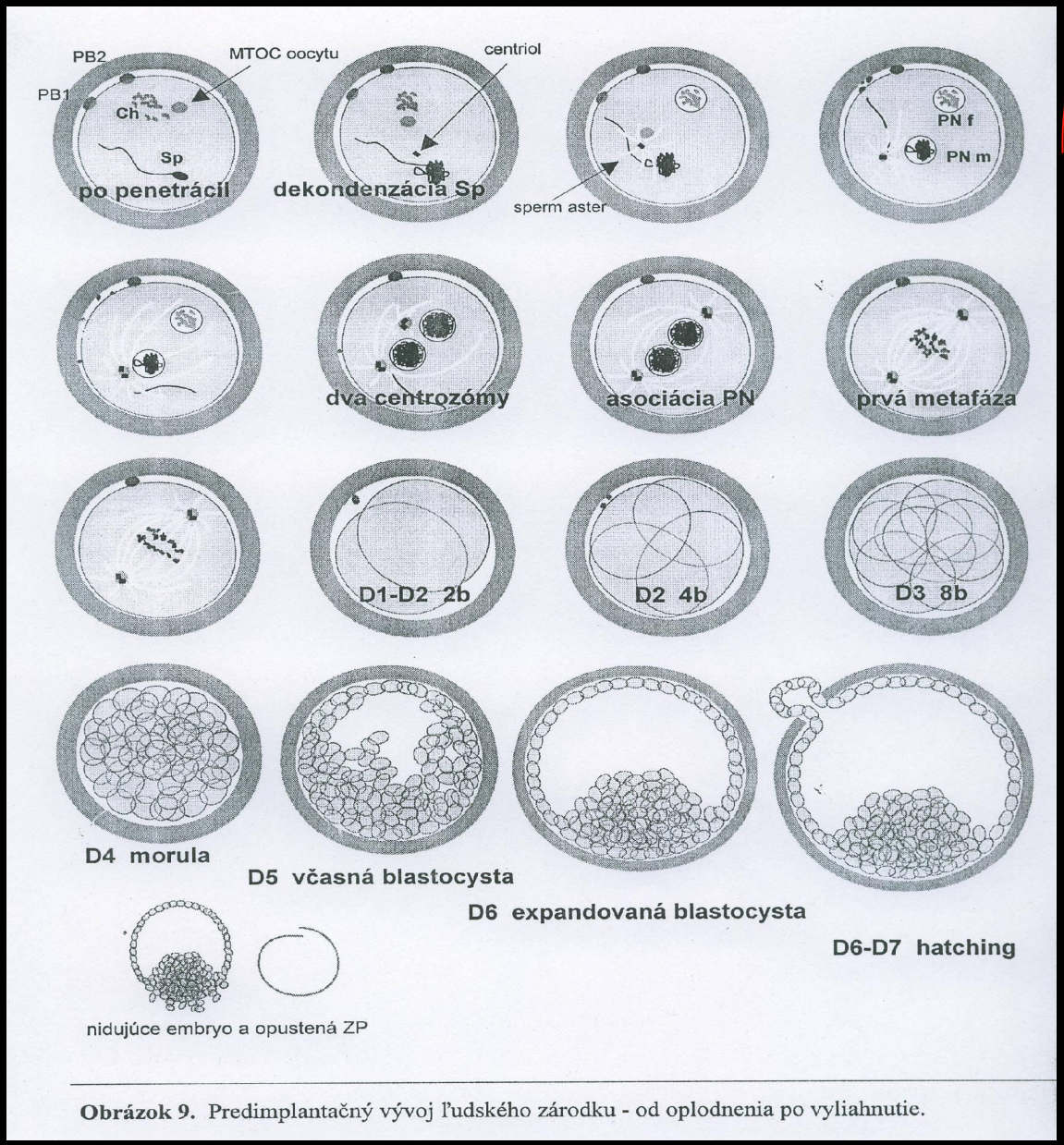
Ideal features shared by zygotes that have high viability:
(1) the number of nucleoar precursor bodies (NPB) in both pronuclei never differed by more than three
(2) the NPB are always polarized or not-polarized in both pronuclei but never polarized in one pronucleus and not in the other
(3) the angle from the axis of the pronuclei and the furthest polar body is less than 50°

Fig 17.1
Ideal features shared by pronuclear embryos that have high viability as described by Tesarik and Greco,[17] Garello et al,[21] and Scott and Smith.[22]

Ideální znaky zygoty

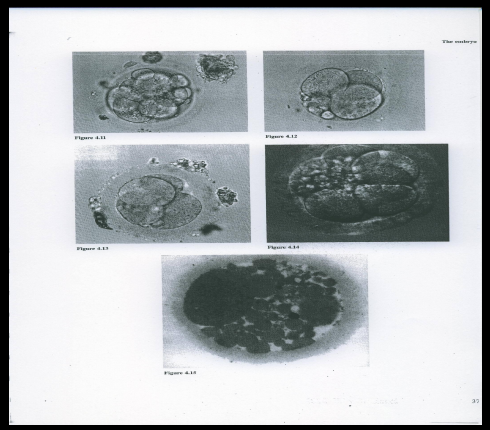
- počet jadérek v obou jádrech není nikdy menší než 3
- jadérka jsou vždy zároveň v obou jádrech buď polarizované nebo nepolarizované
- úhel mezi osou prvojader a pólovými tělísky je menší než 50°

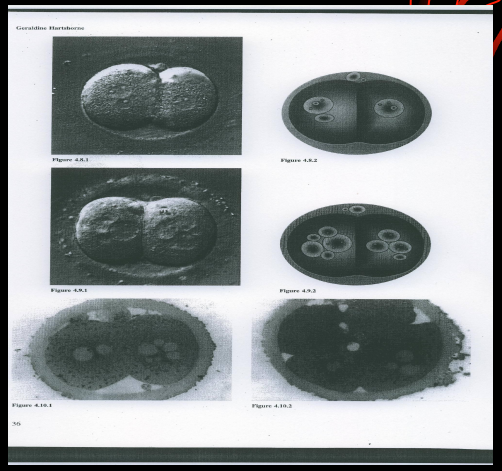
Vývojové schéma od inseminace až po hatchujíci blastocystu

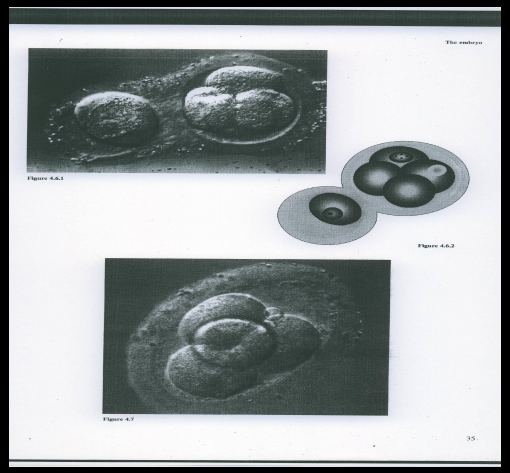


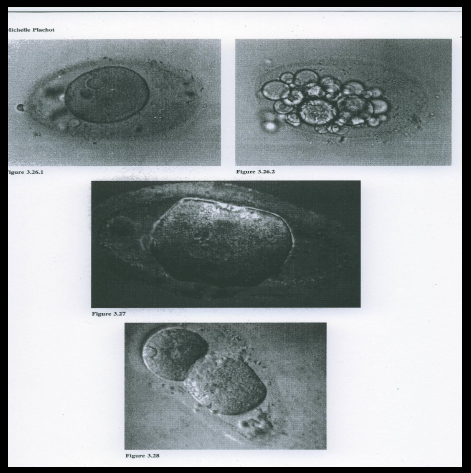
Obrázok 9. Predimplantačný vývoj ľudského zárodku - od oplodnenia po vyliahnutie.

Možné poruchy ve vývoji embryí









Vývoj od kompaktace k blastocystě

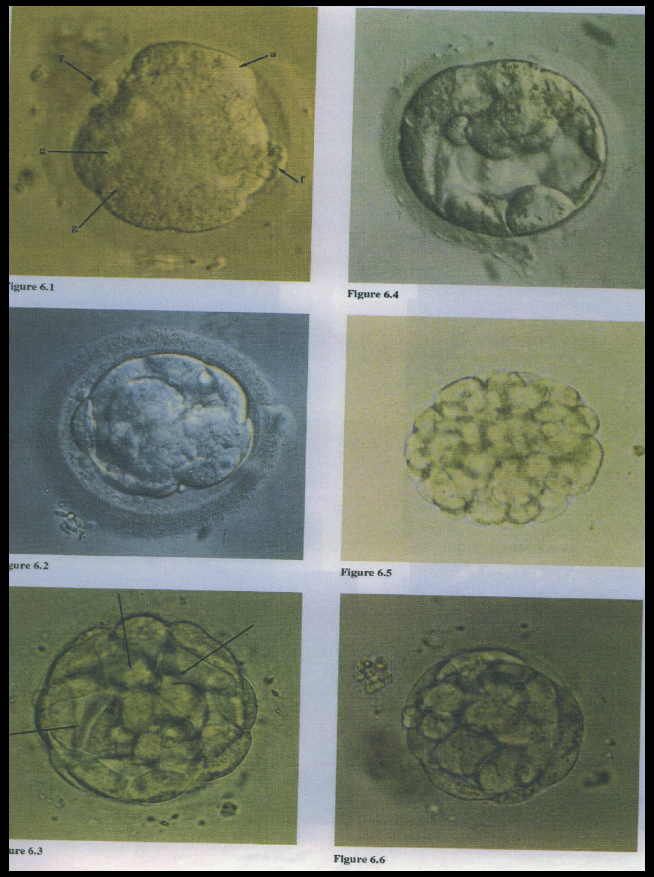


Schéma vývoje blastocysty

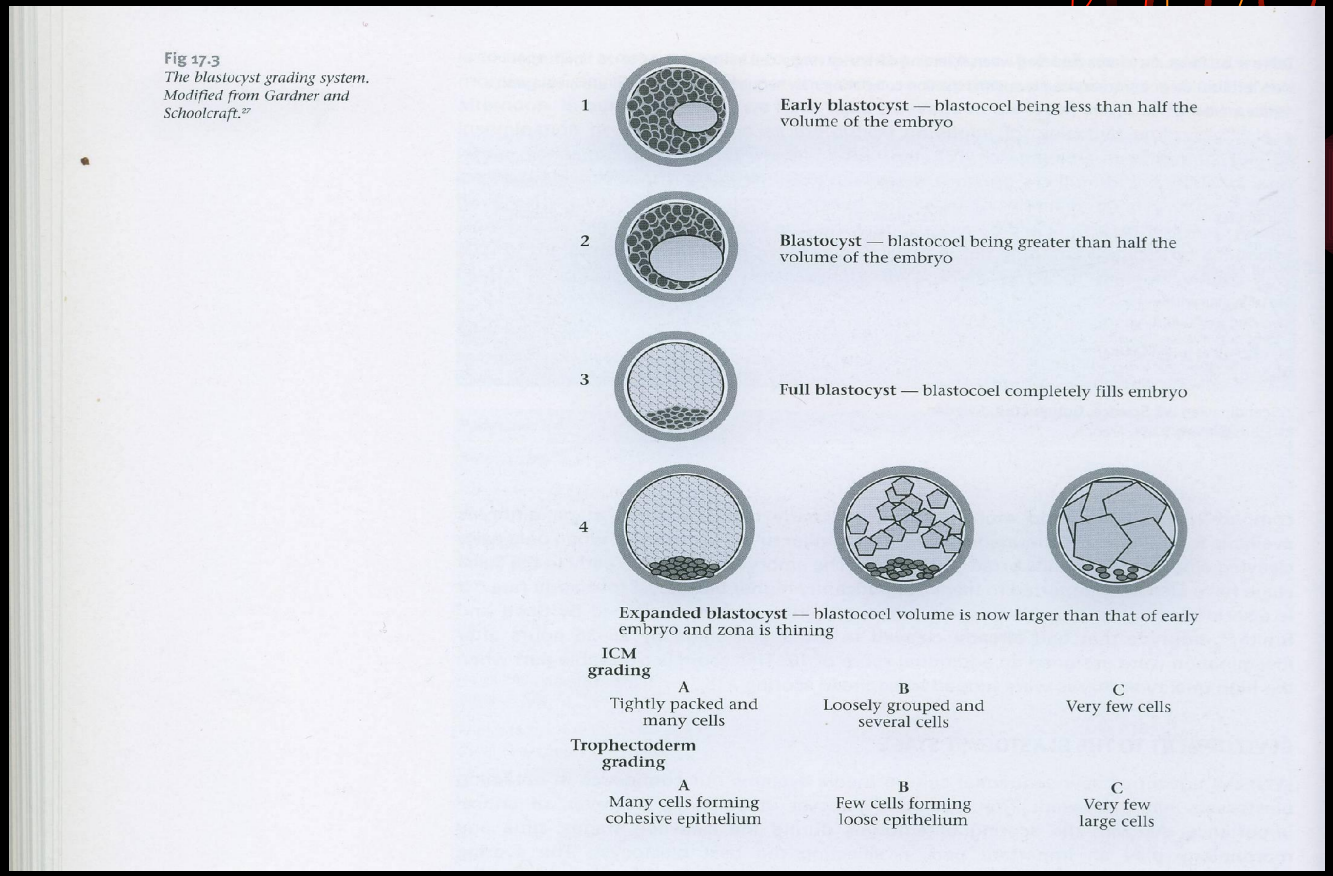


Fig 17.3
The blastocyst grading system. Modified from Gardner and Schoolcraft.[27]

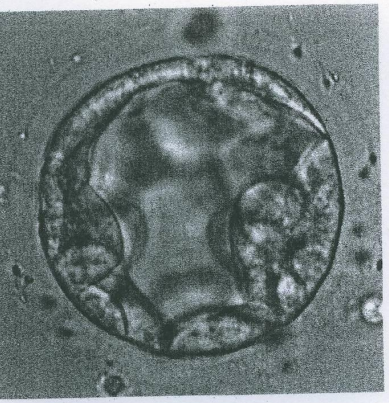


Figure 6.7

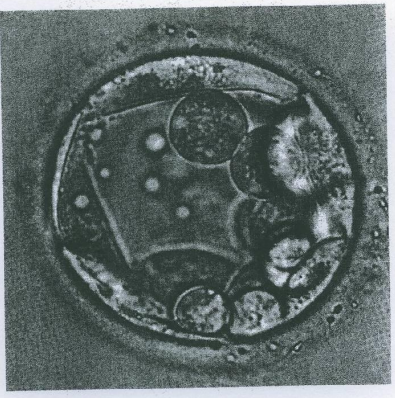


Figure 6.8.1

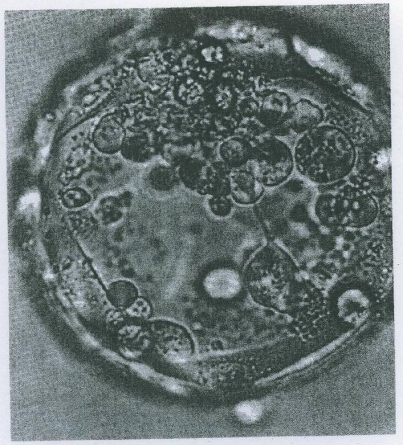
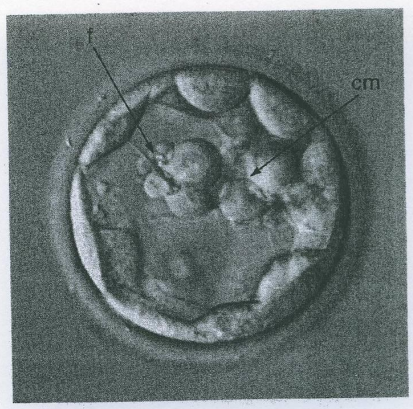


Figure 6.8.2



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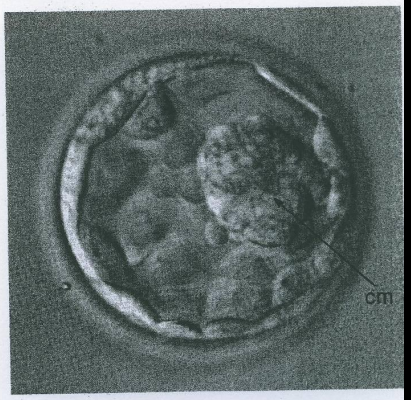

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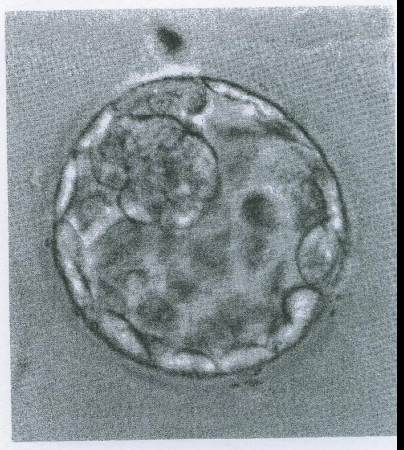


Figure 6.11

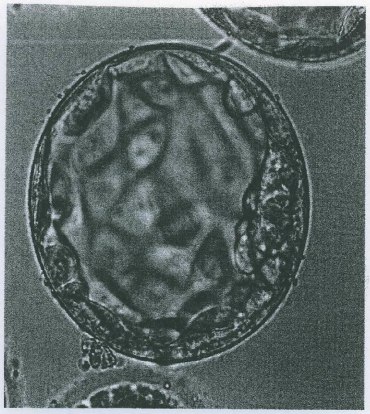


Figure 6.12

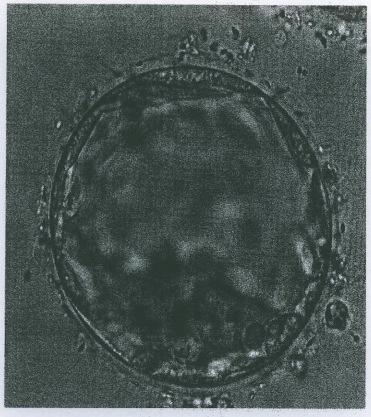


Figure 6.13

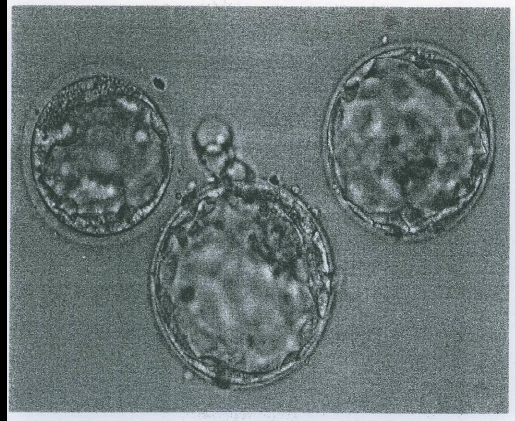


Figure 6.14

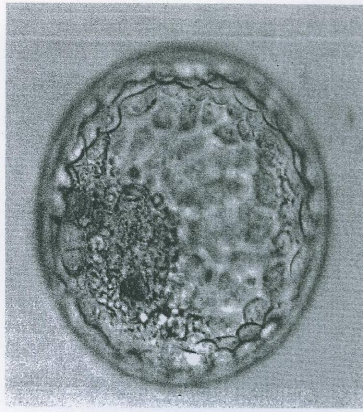
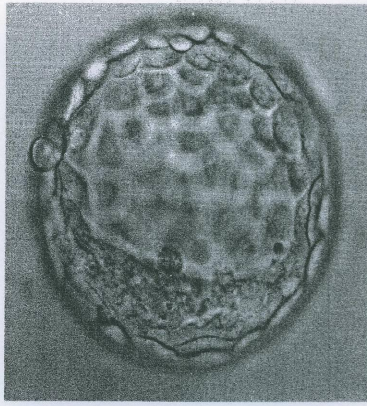


Figure 6.15

Figure 6.16



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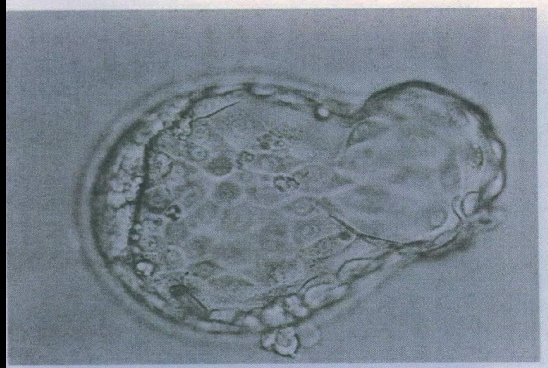

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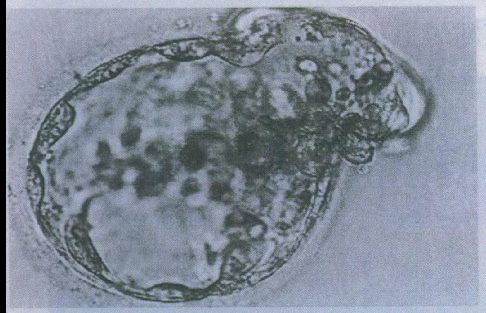

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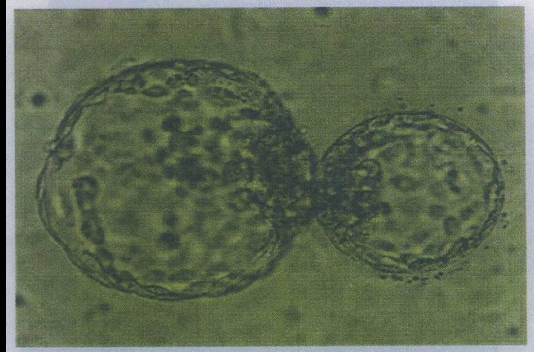

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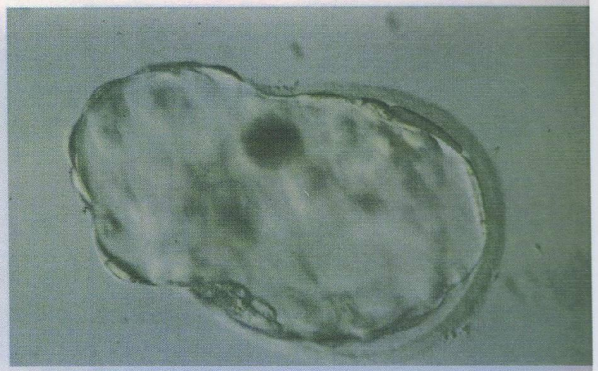

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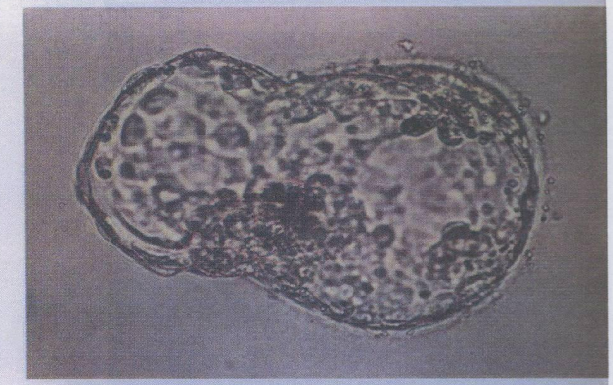

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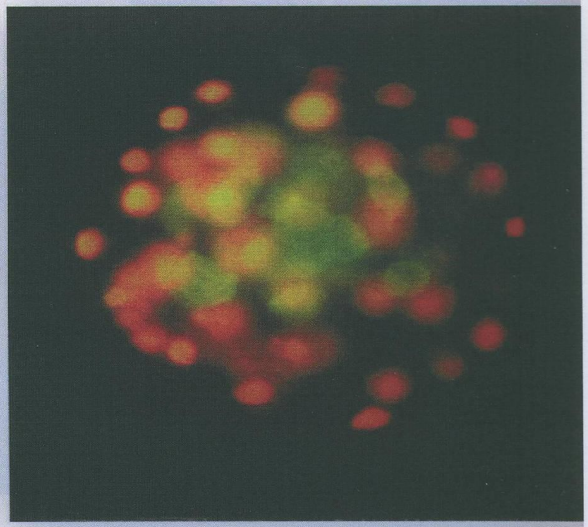

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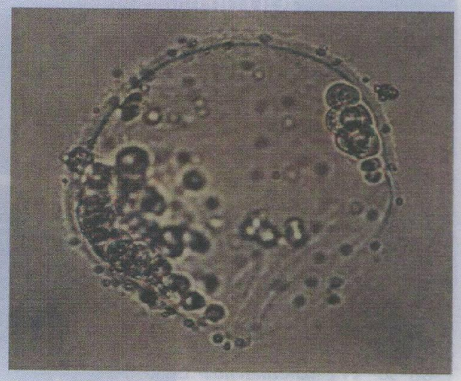

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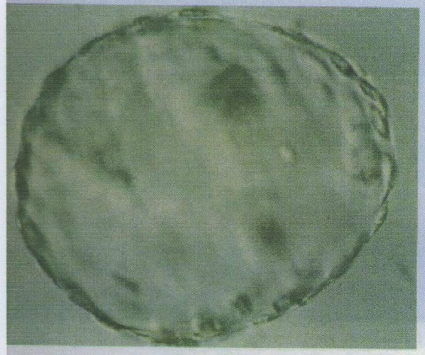

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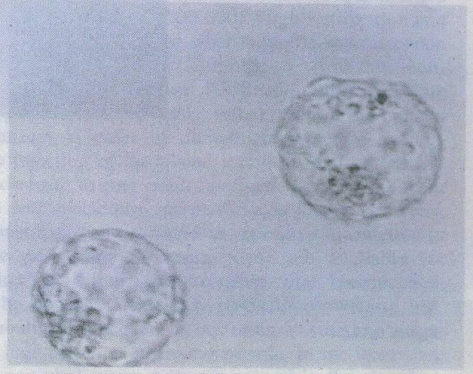

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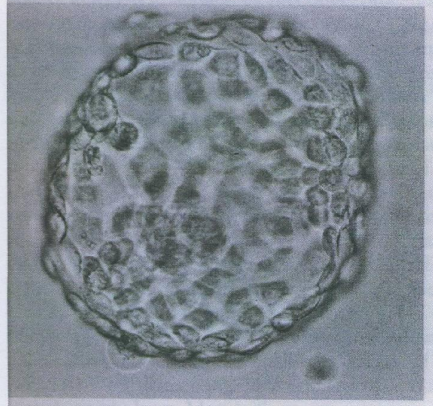

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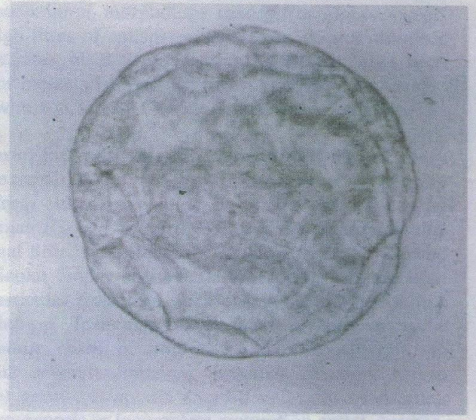

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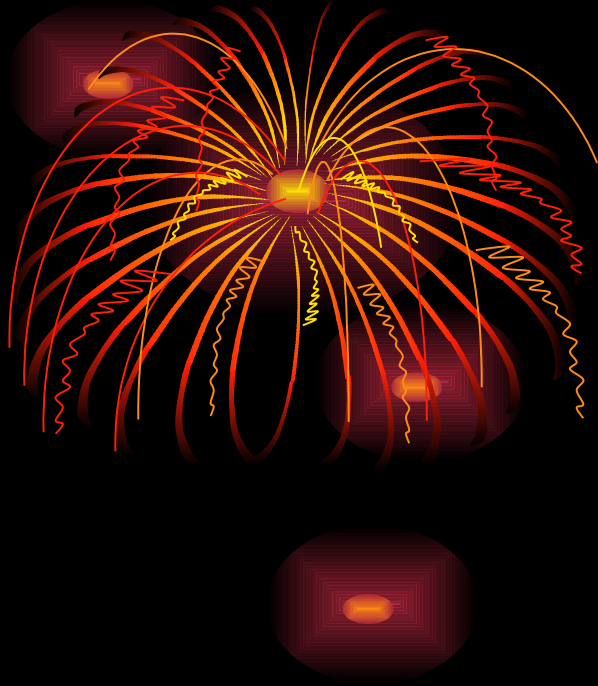


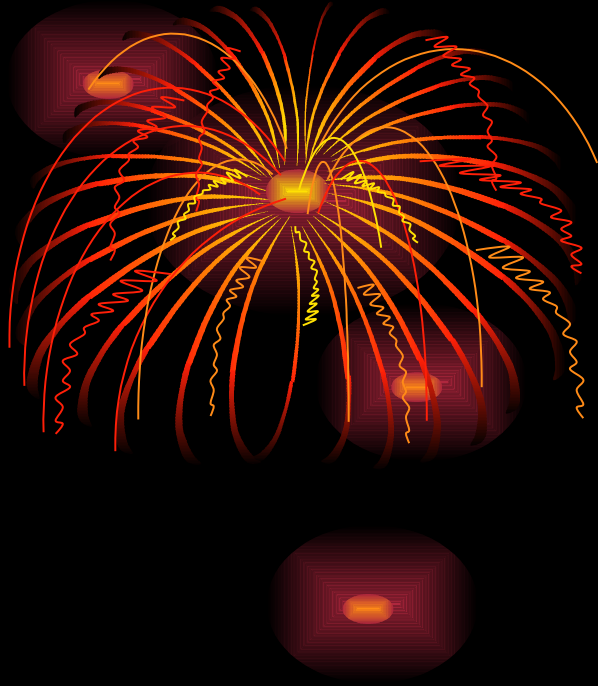
Strategie výběru nejlepšího embrya

multiple step scoring system

Hypotetický plán hodnocení:

- **18 – 19 hod po inseminaci se hodnotí zygoty:**
  - **symetrie prvojader**
  - **přítomnost, rozložení a počet jadérek**
  - **pozice pólových tělísek**

- **25 -26 hod po inseminaci**



- **rýhující embrya ve 2 buněčném stádium**
- **u progresivních zygot nuclear-membrane- breakdown**

• 42 - 44 hod po inseminaci

- počet bastomer rovný nebo vyšší než 4
- fragmnetace nižši než 20 %
- žádné blastoméra s multinukleárním jádrem

• 66 – 68 hod po inseminaci

- počet blastomér větší nebo rovný 8
- fragmentace menší než 20%
- žádné blastoméra s multinukleárním jádrem

•106 – 108 hod po inseminaci

- **Plně rozvinutá dutina blastocysty**
- **ICM z velkého počtu buněk těsně spakovaných**
- **TE početná a kohezivní**

Hodně úspěchů.

Děkuji za pozornost.