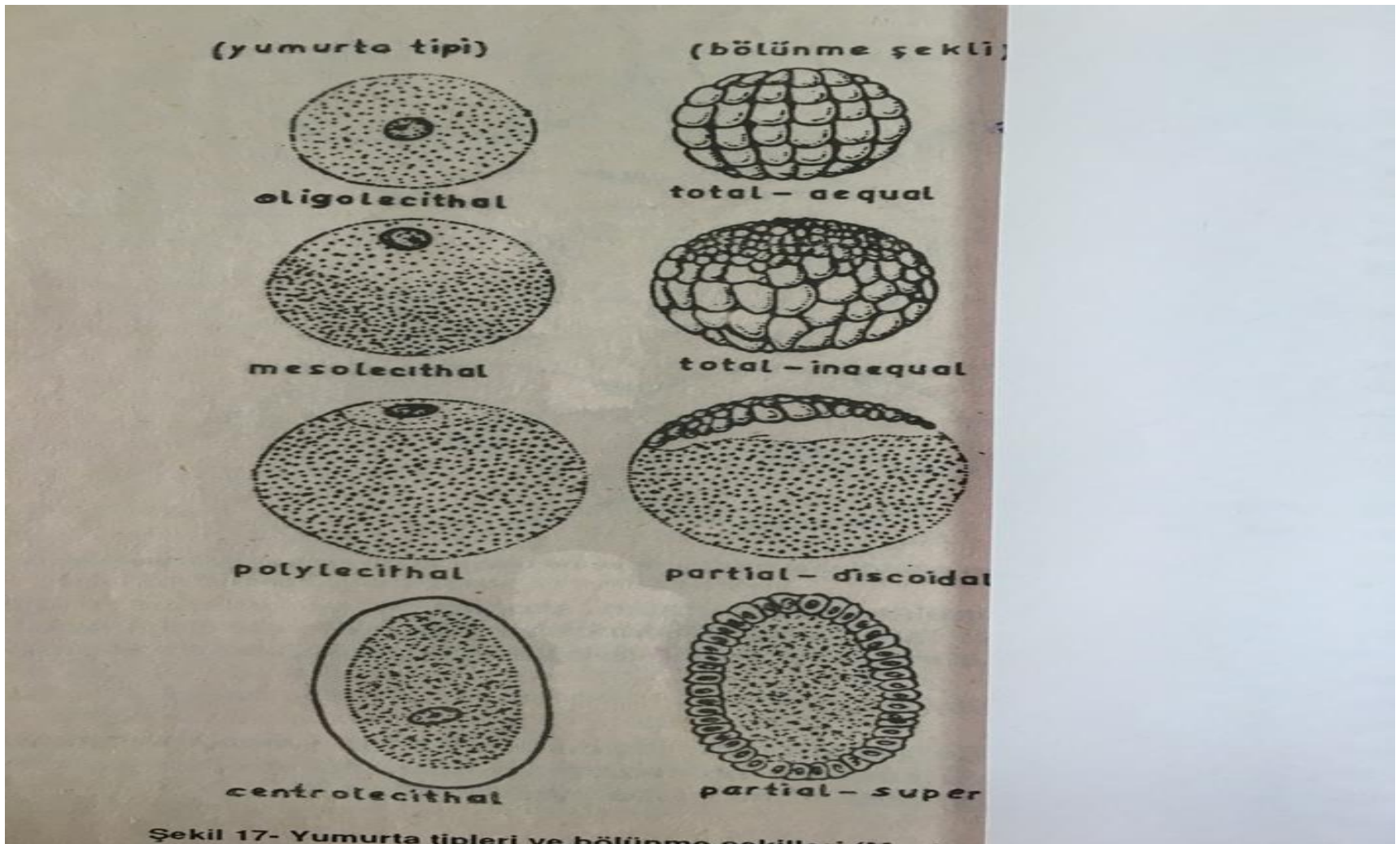


Types of Ovum and Cleavage



- The pronuclei then move towards each other in the centre of the zygote, their nuclear envelopes dissolve, and the chromatin condenses to enter prophase of the first mitotic division. First cleavage is normally seen within 24 hours after ovulation.

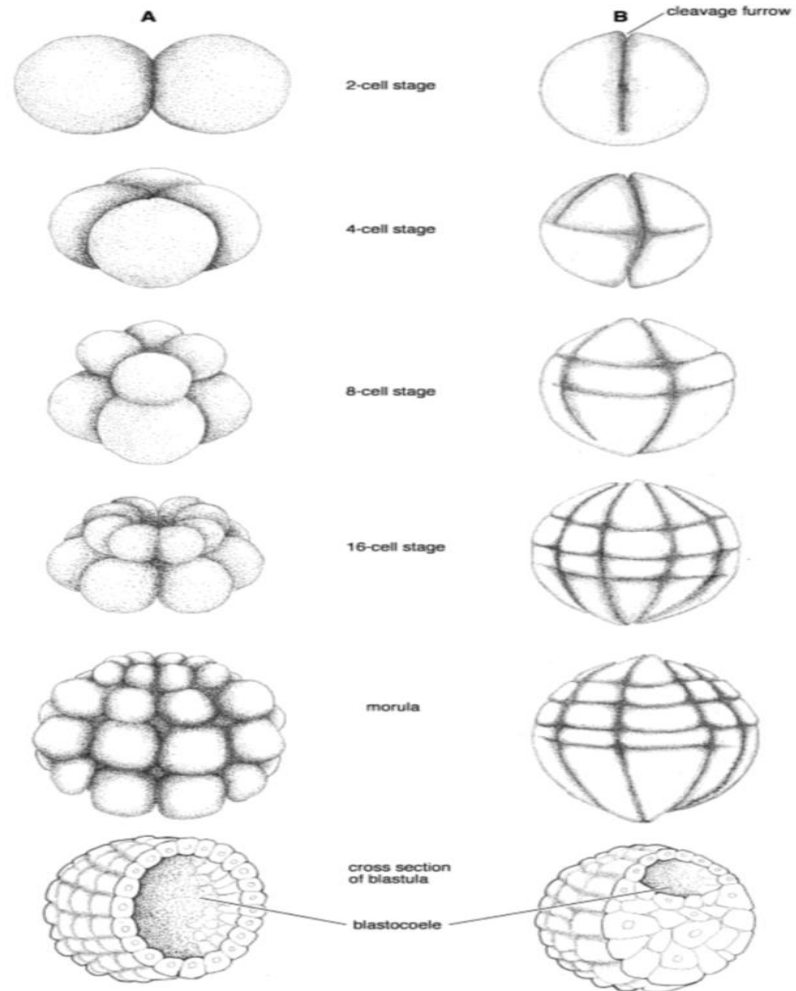


Figure 4.1 Stages of cleavage, from the two-cell stage to the early blastula stage in *Amphioxus*, A, and amphibians, B.

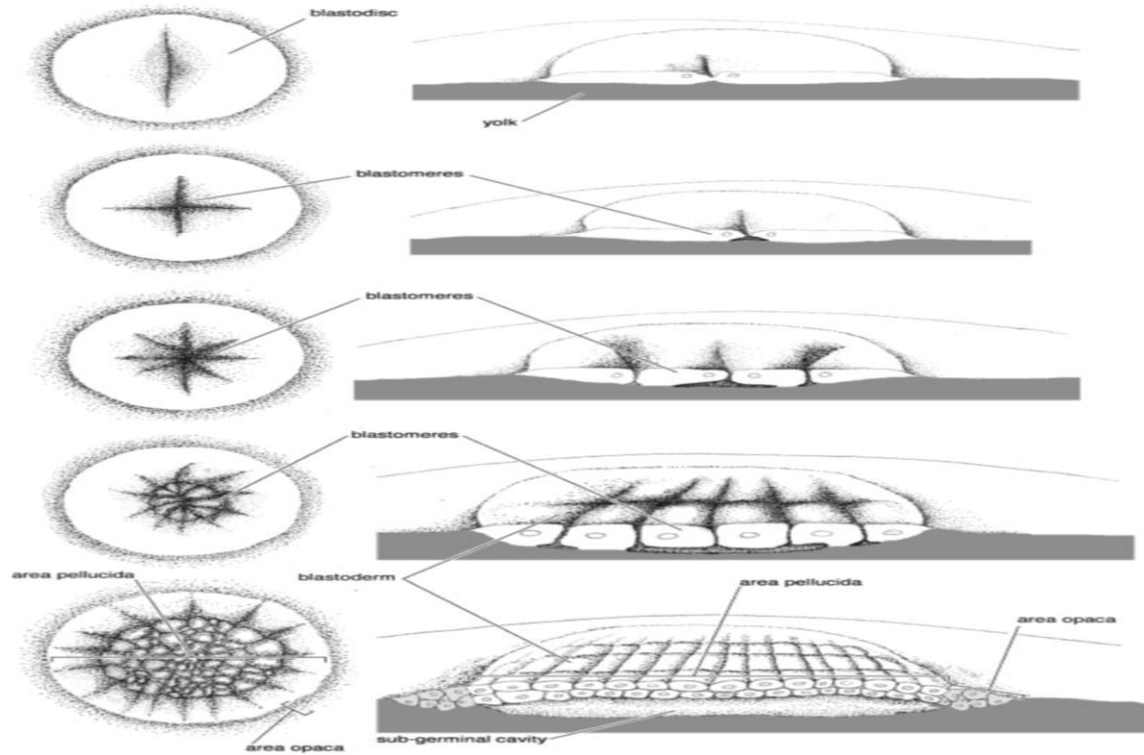


Figure 4.2 Stages of cleavage in the avian zygote from the first cleavage division to the formation of a blastoderm. Blastodisc viewed from above (left), and in cross-section (right).

In vitro fertilization

Why is IVF used?

IVF can be used to treat infertility with the following patients:

1. Blocked or damaged fallopian tubes
2. Male factor infertility including decreased sperm count or sperm motility
3. Women with ovulation disorders, premature ovarian failure, uterine fibroids
4. Women who have had their fallopian tubes removed
5. Individuals with a genetic disorder
6. Unexplained infertility

There are five basic steps in the IVF and embryo transfer process:

1. Monitor and stimulate the development of healthy egg(s) in the ovaries.
2. Collect the eggs.
3. Secure the sperm.
4. Combine the eggs and sperm together in the laboratory and provide the appropriate environment for fertilization and early embryo growth.
5. Transfer embryos into the uterus.

In vitro fertilization

- For in vitro fertilization, the oocytes are co-cultured with spermatozoa separated from fresh or commercially available frozen semen, depending on the species. Efforts to establish new evaluation methods for sperm quality and ability to induce normal embryo development have not become very practical and, in most laboratories, post-thaw motility is still the only test used routinely; the quality of a particular batch of semen is generally judged from the rates of embryo production that it gives.

In vitro fertilization

- Various techniques are used to prepare the spermatozoa, most commonly centrifugation of the spermatozoa in a percol gradient followed by the so-called 'swim-up' separation: progressively motile spermatozoa are capable of swimming up into the more superficial layers of the fluid columns, away from their inactive counterparts

- Capacitation of spermatozoa in the uterus and oviduct is, of course, not possible in vitro. Hence, to promote capacitation in vitro, some laboratories treat the spermatozoa with **Ca⁺⁺ ionophore**, but most use heparin treatment before and/or after addition of spermatozoa to the oocytes.

- To stimulate sperm motility, a mixture of penicillinamine, hypotaurine and epinephrine is commonly added to the fertilization medium, although the efficacy of such treatment has been questioned.

- Two features in particular underline how markedly different are fertilization in vitro and fertilization in the oviduct: first, the spermatozoa/oocyte ratio is approximately 10^3 – 10^4 times higher in vitro than in vivo; second, the oocytes usually remain surrounded by cumulus cells in vitro whereas in the large domestic species these cells are largely shed during or shortly after ovulation in vivo.

- The seemingly feasible explanation that the high number of spermatozoa is needed to overcome the cumulus barrier has not really been substantiated because similarly high spermatozoa/oocyte ratios are required to fertilize cumulus-denuded oocytes.

- Indeed, removing the cumulus cells from the zona pellucida may actually decrease the percentage of penetrated oocytes without increasing the rate of polyspermic fertilization. Attempts to mimic, in vitro, the much lower spermatozoa/oocyte ratio and the cumulus cell investment that prevail in vivo have generally resulted in decreased rates of fertilization and blastocyst formation.

7. Cloning

- ▶ Dolly the sheep - 1997.
- ▶ In theory could be used to increase the numbers of embryos created in an IVF cycle, or to create a duplicate embryo that could be tested for genetic abnormalities and discarded, while its intact “twin” could subsequently be implanted.



Image source:
http://usatoday30.usatoday.com/tech/science/genetics/2006-07-04-dolly-anniversary_x.html

(8.) In Vitro Fertilisation (IVF)

History:

- ▶ Research began in the 1930s.
- ▶ 1st successfully performed on mouse in 1958.
- ▶ IVF with human gametes - pioneered by Robert Edwards and Patrick Steptoe during the 1960s and 1970s -> first IVF baby, Louise Brown (25.07.1978).
- ▶ Initially - hostility to IVF and scepticism about its safety and efficacy.

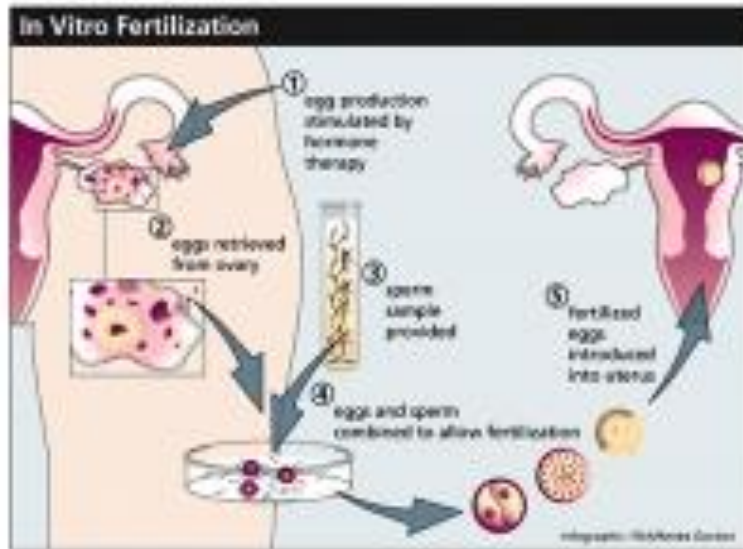


(Image source: <http://www.dailymail.co.uk/news/article-237851/1978-07-25.html>)



(Image source: <http://www.dailymail.co.uk/news/article-237851/1978-07-25.html>)

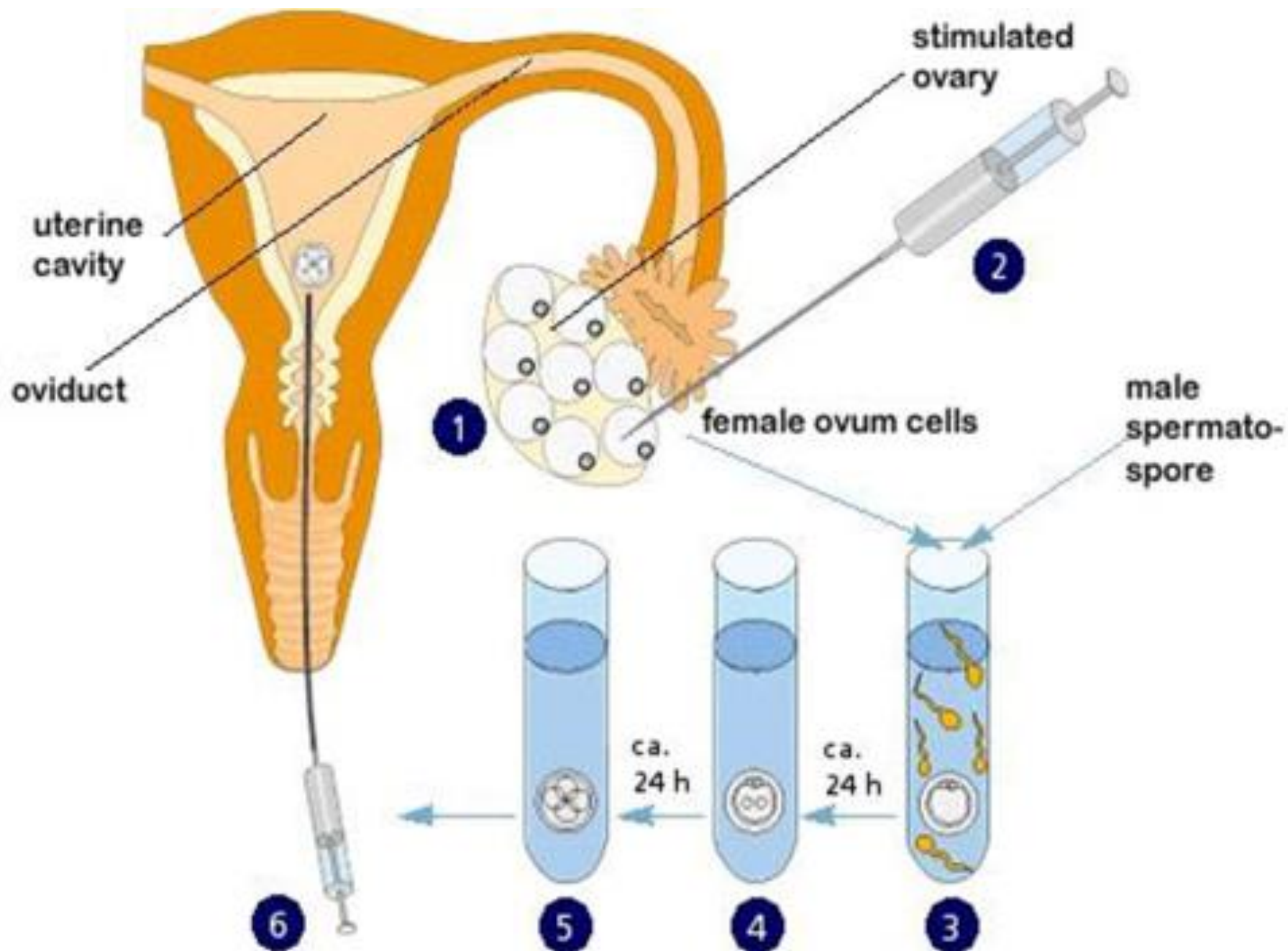
In Vitro Fertilisation (IVF)

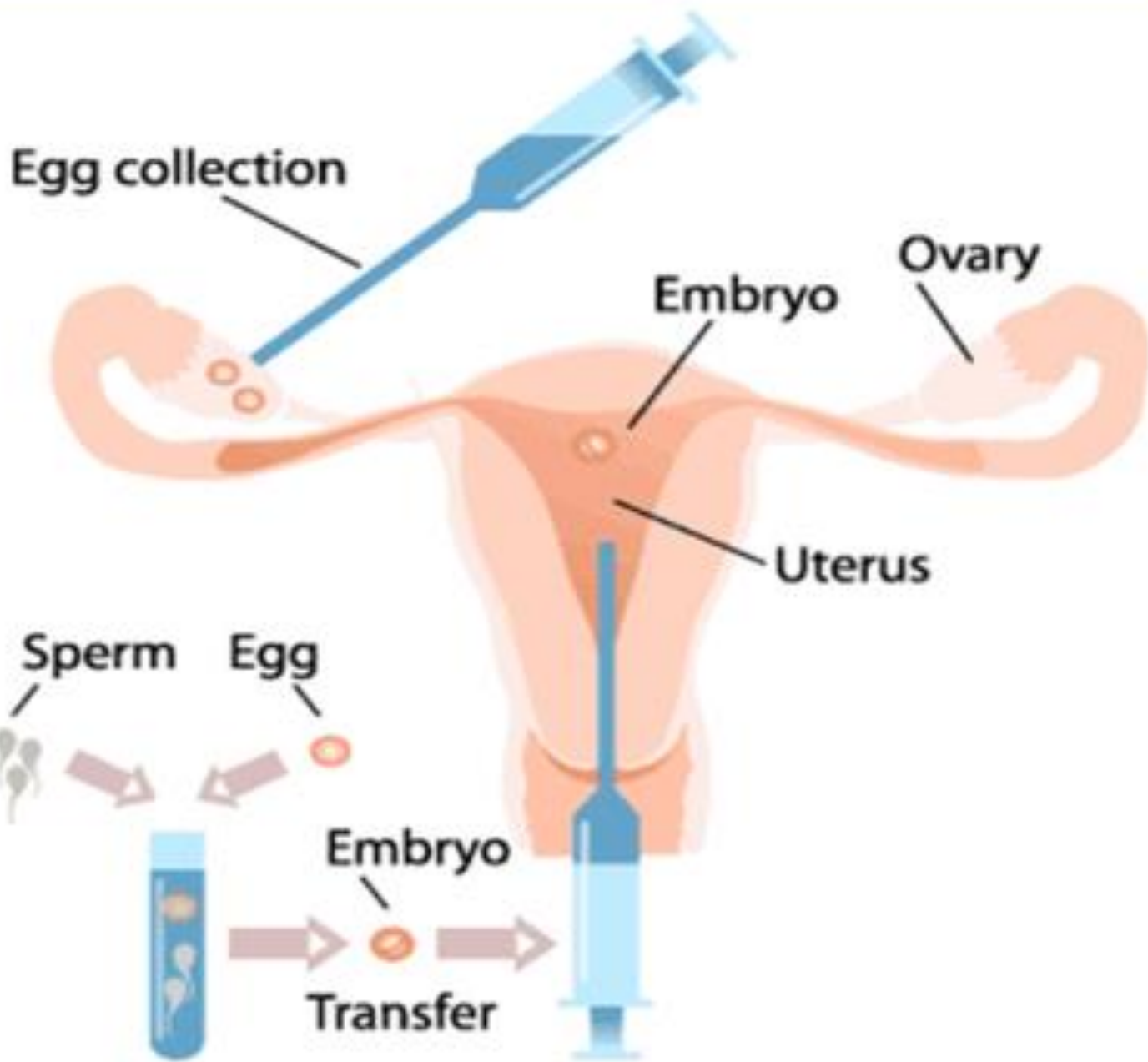


(Image source: <http://www.medicalhealthcare.com/procedure/in-vitro-fertilization/2/>)

Procedure:

1. Commonly hormonal treatment - producing more eggs.
2. Removal of the eggs from the ovarian follicles through laparoscopy or transvaginal aspiration and placing them in a culture that allows them to mature further.
3. Providing of sperm.
4. Putting the mature eggs into a petri dish with sperm (usually from the woman's partner).
5. If fertilisation occurs the resulting zygote(s) may be placed in the woman's uterus, or frozen to be used at a later date.





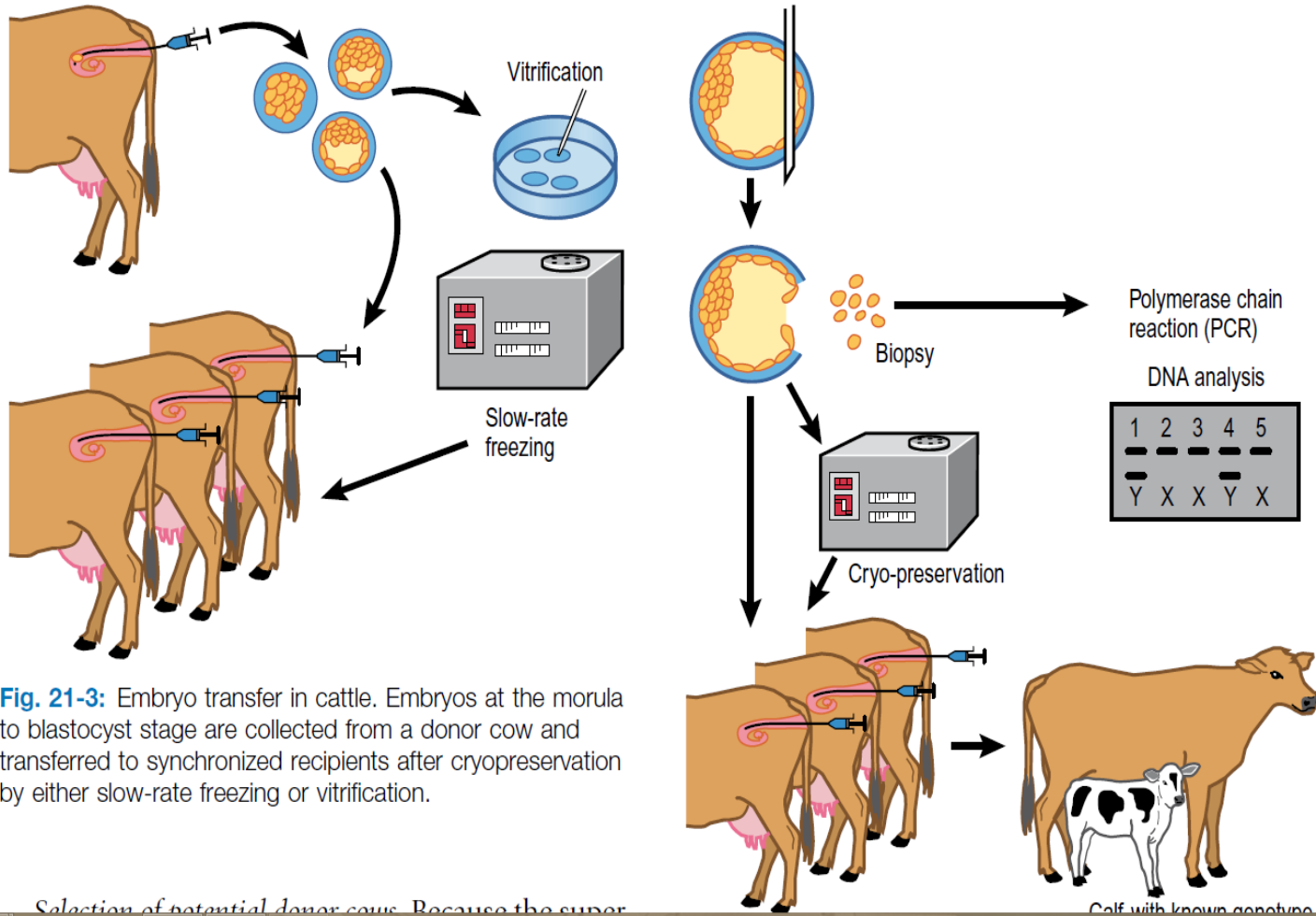
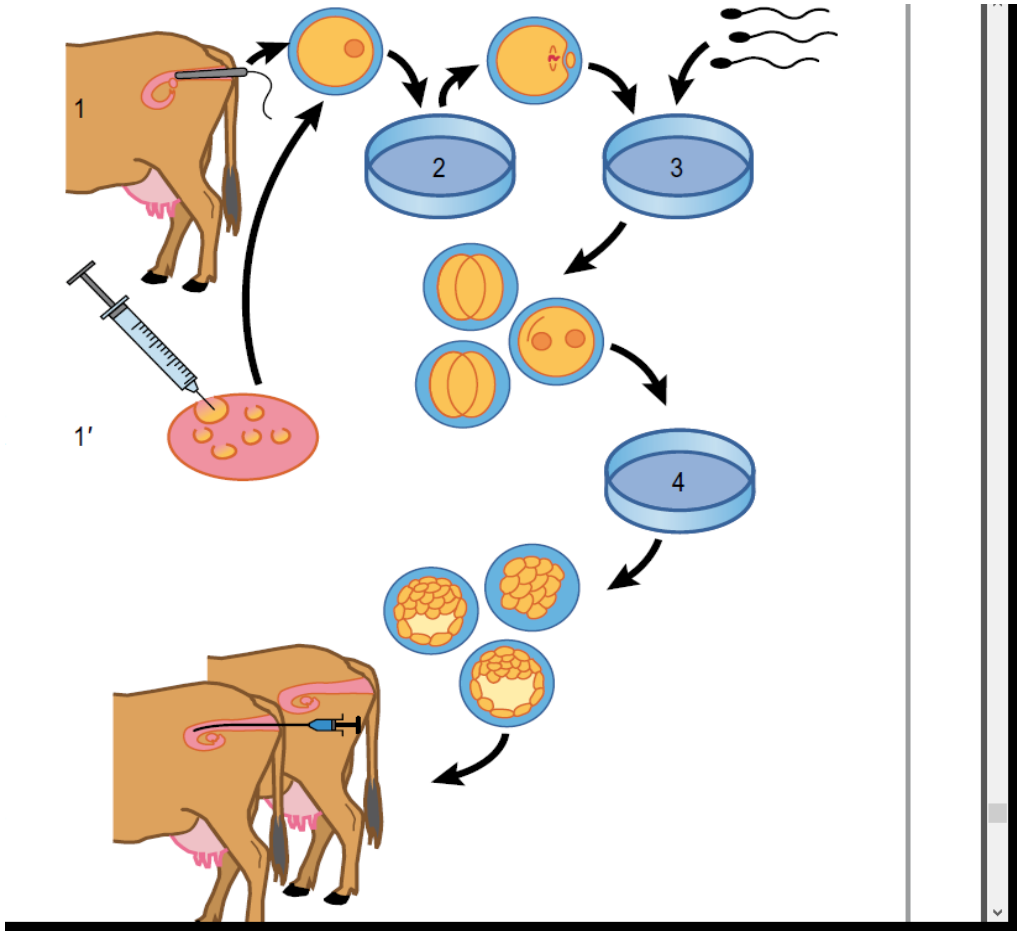


Fig. 21-3: Embryo transfer in cattle. Embryos at the morula to blastocyst stage are collected from a donor cow and transferred to synchronized recipients after cryopreservation by either slow-rate freezing or vitrification.



- REFERENCES:

- [?] Hyttel, P., Sinowatz, F., Vejlsted, M., & Betteridge, K. (2009). Essentials of domestic animal embryology. Elsevier Health Sciences.
- [?] Junqueira, L. C., & Mescher, A. L. (2009). Junqueira's basic histology: text & atlas (12th ed.)/Anthony L. Mescher. New York [etc.]: McGraw-Hill Medical, Chapter 15, pp. 314-354.