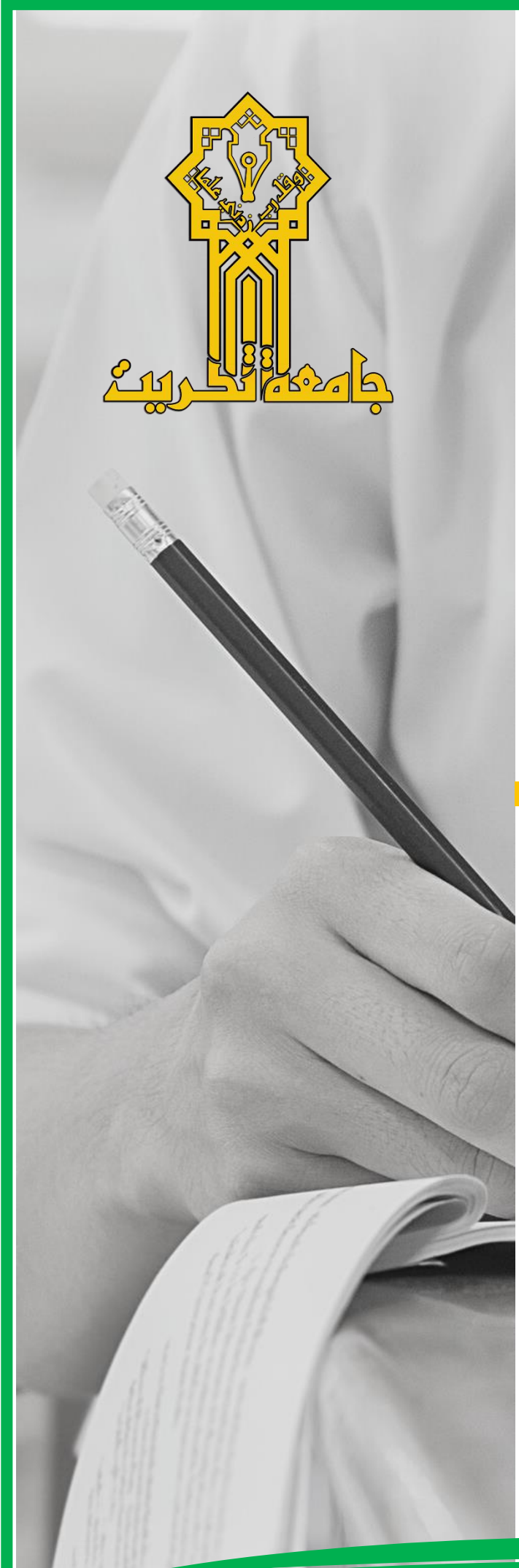

Tikrit University
College of Veterinary Medicine

In vitro embryo production

Subject name: Reproductive techniques

Subject year:5

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SCAN ME
Lecturers link

In vitro embryo production (IVEP)

It is an assistive reproductive technology, main function of IVEP is to provide a medium outside the body (in laboratory) to the embryo is chemically similar to what it is in the female genital canal and suitable for growth. It consists of several stages; maturation of the oocyte and the occurrence of adaptation to the sperm and then successful fertilization.

A cumulus oophorus: layer plays an important role during the growth and maturity of an oocyte where nutrients are transported from the vesicle or the ovarian medium to the oocyte.

Zona pellucida: plays a key role in regulating fertilization and fetal protection.

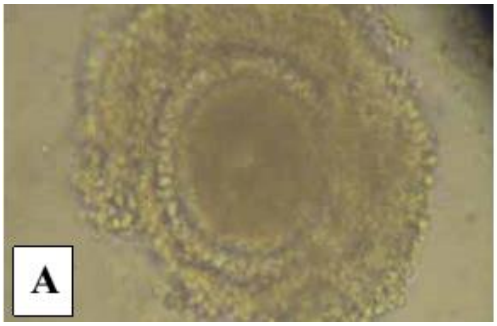
First: Methods of oocyte collection:

1- Surgical procedures.
2- Ovum pick-up (OPU).
3- Slicing.
4- Puncture.
5- Aspiration.
6- Laparoscopic oocyte recovery.
7- Slashing.

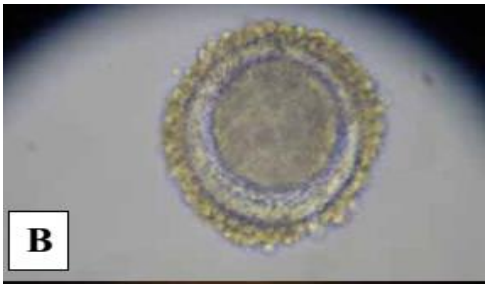
Second: Evaluation of aspired oocyte:

Three type of aspired oocyte can found microscopically:

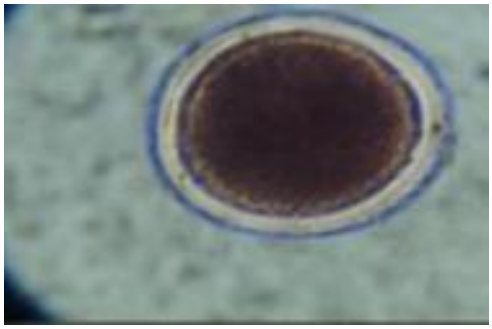
1. Good: oocyte completely surrounded by cumulus cell.



2. Fair: oocyte partially surrounded by cumulus cell.



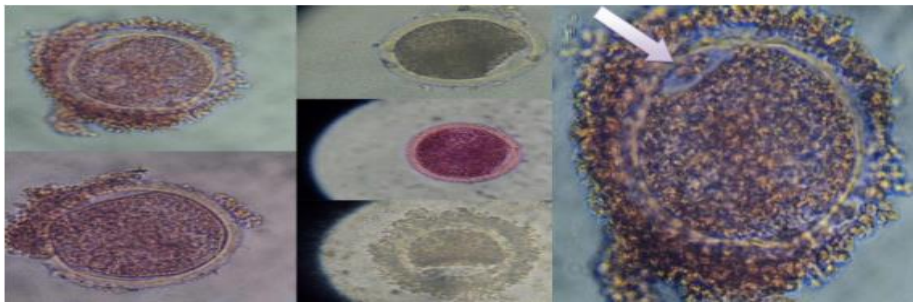
3- Poor: denuded cell.



Third: In Vitro Maturation (IVM)

After collection the oocytes, it should be incubate the ovum in culture media like Tissue culture medium 199 (TCM 199) supplemented with different components for this stage with gonadotropins as LH, FSH or a combination to increases the number of oocytes reaching MII and improves the rate of viable embryos, also estradiol is important. The oocytes should be incubated in 38.5 c and 5% Co2 for 24h with humidity 90%. Then, is evaluated under microscope, the first obvious sign of resumed 1stmeiosis is the dissolution of the nuclear membrane (Germinal Vesicle Breakdown) and extrusion of the 1stpolar body and formation of the second meiotic spindle subsequently occur as the oocyte matures and the surrounding cumulus cells undergo expansion.

Normally more than 90% of metaphase II oocytes is expected to obtain under these conditions.



Forth: Collection of sperm and In Vitro sperm capacitation:

After surgical collecting the tail of the epididymis, put it in dish and inject the cauda of epididymis with Minimal Essential Medium (MEM) and then slicing it by surgical scalpel and then withdraw its content.

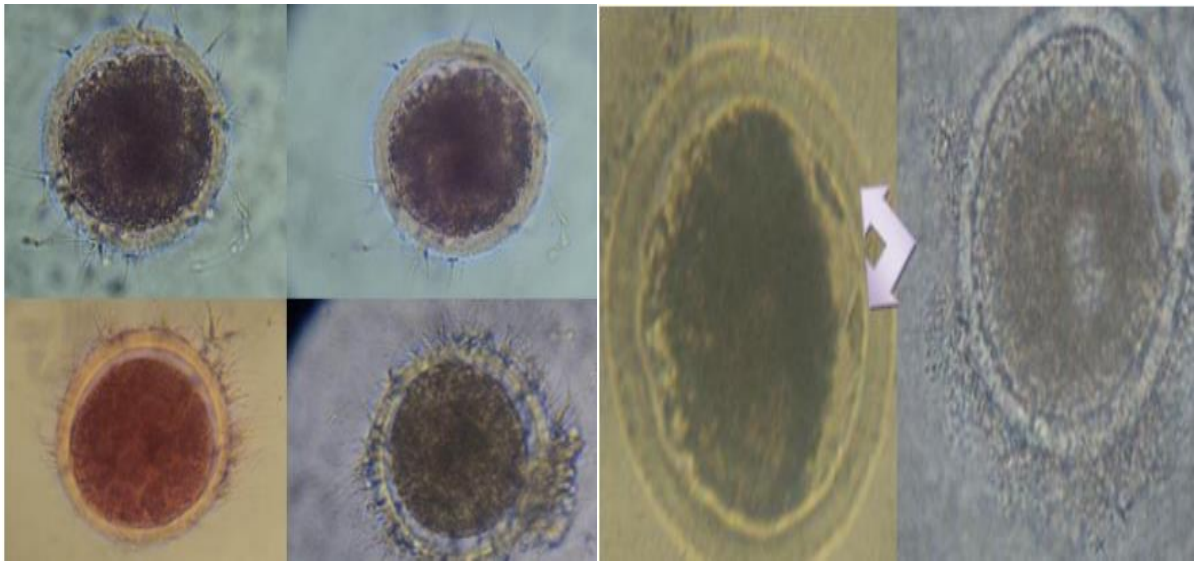
In Vitro sperm capacitation consider a prerequisite for fertilization.

Heparin is one of his Glycosaminoglycans (GAGs) group used for capacitation. The ability to bind the proteins in the sperm plasma and act on the events of physiological changes necessary for acrosome reaction, the presence of bovine serum albumin (BSA) in the medium helps to remove cholesterol and zinc from the sperm membrane and leads to better adaptation. There is a positive correlation between cholesterol depletion from the plasma membrane of the sperm and improved sperm adaptation . The capitation is performed by incubated the sperm in 35c, 5% Co2 for 6 h. After that, the heparin is added to sperm and incubated for 45 m.

Fifth: In Vitro fertilization (IVF)

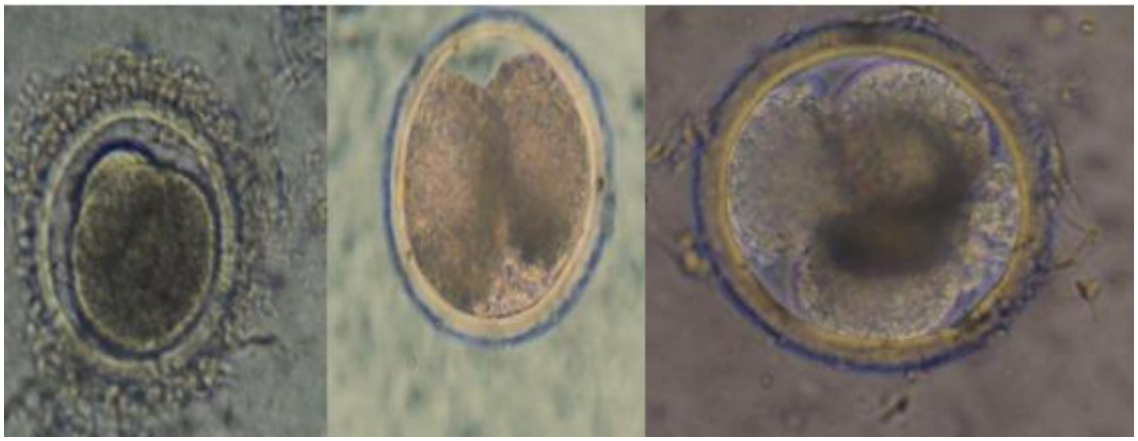
During this stage the zygote will happen and 2nd polar body will appear. The capacitated added to COCs in fertilization medium after IVM.

The osmolarity should be (7.4-7.8), draw about 50% from maturation media and exchanged by culture media with the necessary components for fertilization. The sperm is added to media and then incubated 38.5 c and 5% Co2 for 30 h.



Sixth: In vitro Culture:

The zygote cultured by incubated in 38.5 c and 5% Co2 and substitution of 50% from culture media every 24h. The fertilized ovum of sheep reached the 2-4 cells stage during 24h and to the stage of morula within 120 h and to the stage of the blastocyst in 165 h and to hatching in 216 hours accompanied by the formation of the inner cell mass and trophectoderm. These events are negatively affected by inadequate culture conditions and several strategies have been designed to mimic the female tract in the lab. Different culture media have been tested in small ruminant's embryos; however, Synthetic Oviduct Fluid (SOF) supplemented with BSA.



Culture Systems

Characteristic feature of Simple and complex maturation media

Many culture media like Tissue culture medium 199 (TCM 199), Tyrode's albumin lactate pyruvate (TALP), Synthetic Oviduct Fluid (SOF) and Minimal Essential Medium (MEM) used to providing a favorable environment and nutritional needs of oocyte to grow and develop as if within in vivo. Simple media are usually bicarbonate-buffered systems containing basic physiological saline with the addition of pyruvate, lactate and glucose because it is an important components of the maturation media of oocytes that affect the metabolism and the possibility of evolution. Themedia are usually supplemented with trace amounts of antibiotics
(penicillin, streptomycin, and gentamycin). Complex media contain, in addition to the basic components of simple media, amino acids, vitamins, purines and other substances that mainly in the concentrations found in serum, also gonadotropins; steroids; growth factors, cytokines and antioxidants should be added. It has also been essential to management of an array of factors such as pH, osmolarity, temperature, gas phase, humidity and the timing of maturation.

Hormones:

Not only does maturation improve, but it encourages oocyte to divide earlier in the stage 4-8 cells. FSH is necessary for the expansion of cumulus cells and the maturation of oocyte. Estrogen is of great importance because of its effect on maturation and fertilization of oocytes, also pay a role in maturation of cytoplasm. Also hCG used to increase maturation rate.

Effect of Serum Supplementation:

Serum like bovine serum albumin (BSA), fetal calf serum (FCS), superovulated cow serum (SCS) and oestrus cow Serum (OCS) provides beneficial factors including energy sources, amino acids and vitamins, proteins, growth factors, hormones and other active substances and there may be specific effects of serum components as growth factors that act on improve the rate of maturation of oocytes and thus their i mportance in

improving the development of embryos after IVF, also is very important to prevent the hardening of the transparent area. Additionally, it's contain an anti-oxidant that decreasing oxidative stress. As well as provides a source of albumin that regulate the osmolality, but a serious
risk is contaminated with viruses.

Role of CO2: embryo shows better development, and implantation potential, if the oxygen tension is reduced from that in normal air. Gaseous CO2 dissolves in solution to produce carbonic acid, which reaches equilibrium with the amount of dissolved bicarbonate.