**IVF – In Vitro Fertilization**

 In vitro fertilization the egg is fertilized by sperm in a test tube: then implanted in the uterus .In vitro some of the variables affecting development competence of oocytes are:

 1-The age of the females supplying the oocytes.

 2-Their health and environmental stress, such as heat stress,

 3 – The size and maturity of follicles.

 4 – The size of the oocyte.

 5 – The presence and interaction of cumulus cells with the oocyte.

 6- The conditions of oocyte maturation, such as temperature, pH and gas environment.

 7 – The number of cohort oocytes.

 8- The presence of cumulus cells and cell growth factors in the culture media.

 In July 22 , 1978 , Louise Brown was born , the worlds first baby to be born after conception outside of the human body .

**Application of IVF Techniques**

1 – A significant increase of embryos from high genetic value females because oocytes can be recovered from prepubertal, pregnant and even dead or slaughtered goats

2 – IVF provides an excellent source of low cost embryos for basic research and embryo biotechnology studies (nuclear transfer, transgenesis, embryo sexing and stem cells)

3 – IVEP is used in cattle, pigs, sheep and goats to produce offspring from subfertile males and female

4 – Its applications could be for females possess abnormalities in their reproductive tracts ( ovarian adhesion or blocked fallopian tubes )

5 – IVF is also used for females that are terminal ( age , accident, disease)

6 – IVF still provide opportunities to use relatively low numbers of sperm to produce viable embryos. This allows for the utilization of high value semen and may provide significant opportunities when they are coupled with gender separated semen.

7 – All kinds of embryo research need high number of embryos for manipulation

**How is In Vitro Fertilization Done**?

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

**Step 1:** [Fertility medications](https://americanpregnancy.org/infertility/infertility-medications/) are prescribed to stimulate egg production by hormones .

**Step 2:** Eggs are recovered through aspiration from ovary .

**Step 3:** collection of semen by electro ejaculator or from tail of epidydimis from slaughter house , which is prepared for combining with the eggs.

**Step 4:** In a process called insemination, the sperm and eggs are mixed together and stored in a laboratory dish to encourage fertilization. In some cases where there is a lower probability of fertilization, [intracytoplasmic sperm injection](https://americanpregnancy.org/infertility/intracytoplasmic-sperm-injection/) (ICSI) may be used.

 Through this procedure, a single sperm is injected directly into the egg in an attempt to achieve fertilization. The eggs are monitored to confirm that fertilization and cell division are taking place. Once this occurs, the fertilized eggs are considered embryos.

**Step 5:** The embryos are usually transferred into the uterus

**Oocyte collection**:

1-aspiration.

2-slicing.

**Tissue culture media :**

Like, MEM and TCM Media 199

**Oocytes evaluation**

After Collected the oocytes by the four methods, these oocytes are subject to evaluation in regarding to the arrangement of cumulus cell surrounding oocytes as well as status of cytoplasm as:

1- Good grade: when the oocytes with many layers of cumulus cells and a transparent, homogenous and uniform cytoplasm.

2- Fair grade: when the oocytes with less compact cumulus cells with transparent, less homogenous (some granules may found) and uniform. 3-Poor grade: when the oocytes with mild or absent cumulus (denuded) with dark and granular cytoplasm.

After grading and quality evaluation of the collected oocytes, the only good and fair grades oocytes were subjected to the process of *in vitro* maturation (IVM), oocytes after grading were moved to another Petri-dishes containing maturation medium , by aspiration with an automatic micropipette, re-examining the dishes after these ova transport, and to be sure that all selective ova were transport.





Fig. 5: Different grade bovine Oocytes, A. good+ grade, B. good – grade, C. fair grade D. Poor grade oocyte.

**Oocytes maturation**

Good and fair grade oocytes were only subjected to the process of maturation. Maturation medium was equilibrated for two hours in CO2 incubator before oocytes added; 5-6 ml of the previous medium put in glass petridish then oocytes added later. Medium with oocytes was incubated at 38.5°C in CO2 incubator supplied with 5% CO2 tension and 95% humidified air for 27-30 hours, matured oocytes were examined under inverted microscope, **degree of maturation evaluated depending on the entrance of the matured oocytes to the second metaphase (MII) were assessed and observed by the extruding of the first polar body and the degree of the cumulus oocyte complex expanding in which there were three degrees for assessment of oocytes maturation inregarding to these expanding.**

**Maturation and capacitation of caudal spermatozoa**

Epididymal caudal spermatozoa was prepared by aspiration , 2-3ml MEM was loaded in 3ml plastic syringe connected to 18 gauge needle injected then aspired from the cauda more than one time. The sample was evaluated under light microscope for individual and massive motility, individual motility less than 60% was rejected. Spermatozoa samples were incubated in 5% CO2 incubator at 39°C for 4 hours for sperm maturation, presence of distal protoplasmic droplet with head to head sperms attachment were regarded as sperm maturation criteria. Spermatozoal sample might be subjected to the following process the spermatozoa capacitation in which spermatozoa gained its ability to penetrate the zona pellucida of matured Oocytes for fertilization (IVF). The process was prepared by added 50IU of heparin to the matured spermatozoa, and then incubated for 45-60 minutes in CO2 incubator at 39°C, the plastic syringe contained the later mixture was arranged in the incubator in semi-oblique position.

***In vitro* fertilization and embryo production**

Capacitated spermatozoa must be examined before mixed and added to the Petri dish which contained the matured oocytes, diluted spermatozoa must be prepared to yield 1-2 X 106 spermatozoa. Gametes mixture was incubated at 5% CO2 level at 38.5°C and 90% relative humidity for 28-30hrs. Fertilization media supplemented with, LH, FSH, BSA, FCS, antibiotics and antifungal preparation. Developed embryos must be examined and evaluated each 24hrs; embryos showed no progressed signs

Of development must be discarded, all developed embryos must be evaluated and all results must be recorded.