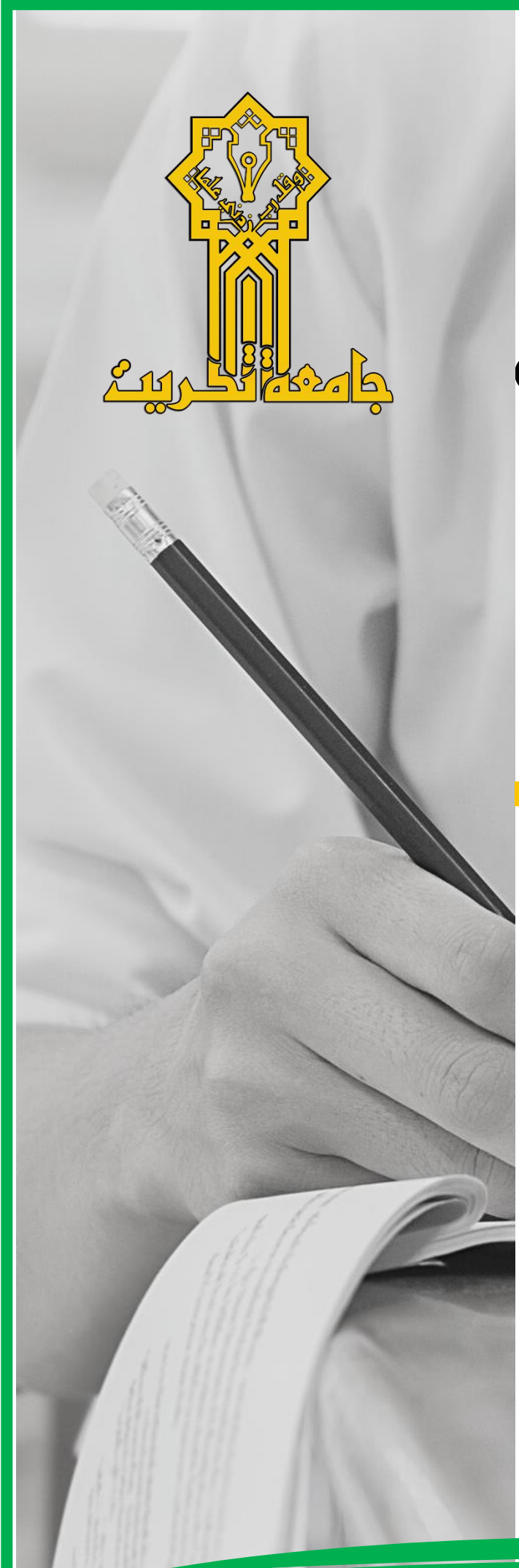

Tikrit University
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Oocyte collection

Subject name: Reproductive techniques

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Oocyte collection

In recent years, new knowledge in the field of assisted reproductive technologies (ART), has allowed researchers and practitioners to reach new hallmarks in oocyte and sperm in vitro competence. Gamete competence is the ability to undergo successful fertilization and develop a normal blastocyst that is capable of implanting in the uterus and generate viable offspring. Many researchers are focused on identifying cellular and molecular markers to select the most competent oocyte and spermatozoon to produce embryos with higher implantation potential.

Additionally, given that the most frequent source of ovaries is slaughterhouse-derived animals, many important factors that influence oocyte quality, such as age of the donor, the stage of the estrous cycle, nutritional status, genetic potential, presence of a reproductive disorder.

Morphological and Visual Markers for the Selection of the Best Oocytes:

1- **Ovarian Morphology:**
   During the retrieval of oocytes from slaughterhouse material, the collection of ovaries based on the presence or absence of estrus cycle structures, i.e., presence or absence of follicles and corpus luteum (CL).
2- **Follicle Size:**
   One of the most used criteria to obtain competent oocytes is the size of the follicle. Research over the past decades indicates that bovine oocytes gain competence at late stages of the follicular phase, when signs of atresia are observed for the first time, such as a slight expansion in the outer cumulus layers and some cytoplasmic granulations.
3- **Morphology of the Cumulus–Oocyte Complexes:**
   The quality of COCs can be influenced by multiple factors, both intrinsic and extrinsic. Intrinsic factors include breed, age, reproductive status, metabolic and nutritional status, hormonal levels, and stage of the estrous

cycle, whereas key extrinsic factors include the timing between slaughter and oocyte withdrawal from the ovary, morphology and methods of collecting the COCs, storage temperature of the ovaries, collection media, and micromanipulation skills of the operator.

4- **Lipid Content:**

The morphological appearance of the ooplasm commonly assessed to select the oocytes is influenced by lipid content in livestock species, such as cattle, pigs, and horses. Lipids, in the form of lipid droplets (LDs), are signaling molecules with important roles in oocyte maturation and competence acquisition. In the late stage of oocyte maturation and during preimplantation development, endogenous oocyte lipids work as an energy source and as a lipid factory for energy reserve.

5- **Cumulus Expansion and Oocyte Size:**

Another parameter that is often used as an indirect indicator of oocyte quality is the degree of cumulus expansion following maturation, typically after 20 to 24 h of culture in an in vitro maturation environment. Grades 1 to 3 (sometimes 4) are attributed to increasing degrees of expansion:

a- modest expansion, characterized by few morphologic changes compared to before maturation.
b- partial expansion.
c- complete or almost complete expansion.

Methods of oocyte collection:

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

Ovum Pick Up in Cattle

The Ovum Pick Up technique consists in the transvaginal removal of the oocytes by aspiration of the ovarian follicles with the aid of an ultrasound probe. This procedure is absolutely harmless to the donor, the time taken for the collection is 15-20 minutes during which the donor is contained in a cattle crush.

Slicing:

ovaries were placed in a graded plastic Petri dish containing a saline solution and were chopped into small pieces with a surgical blade. The cumulus-oocyte complexes (COCs) were selected from the saline solution.

Puncture(slashing):

follicles visible on the surface ranging from 2.0 to 6.0 mm in diameter were punctured with an 18-g needle. The COCs were selected from the follicular fluid.

Aspiration:

the follicular fluid from surface follicles (2.0 to 6.0 mm) was aspirated through a sterile 18-g needle attached to a 5 ml syringe containing a sterile saline solution. Aspirated contents were expelled into a fresh Petri dish containing the saline solution and COCs were selected from it.

Aspiration

Slicing

slashing