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Study of Caprine Oocyte by Intravaginal Culture with Embryo Development

Sadeq Jaafer Zalzala
Department of Theriogenology, College of Veterinary Medicine, University of Baghdad, Iraq

Abstract

This study conducted in Baghdad University/College of Veterinary Medicine. Twenty ovaries collected from slaughtered does and 50 oocytes were aspirated from ovarian follicles. Subsequent submitted to intravaginal maturation. The number of mature oocytes reached 18. Followed by *In vitro* fertilization by mixing matured oocytes and sperms which were aspirated from epididymis of testes in the epindroff tube, then incubated intra-vaginal of doe. Microscopical examination revealed deferent stages of embryo development, 2-cells, 4cells,and over 8-cells in the time 24, 48, and 72 hours respectively. This study showed that intravaginal culture is good technique for field fertilization without CO_2 incubator.

Keywords: intravaginal culture, tail of epididymis, progesterone sponges, caprine

Introduction

Biotechnology is one of the most fast growing scientific disciplines of the twenty-first century. Recent advances in technology, nuclear biology and genetic engineering have made possible a wide range of new technologies and products which provide scientists with a spectacular vision of the design and function of living organisms and provide technologists, including reproductive biotechnologist, with the tools to implement exciting commercial applications (Abu Nasar *et al.*2008).

Reproductive biotechnologies could be an approach to generate transgenic goat for the propagation of useful genetics Wang *et al.* (2002) or gender pre-selection (Hamano *et al.*1999). These goats could be used as founder animals for the production of recombinant protein in their milk such as pharmaceutical proteins for the treatment or prevention of human diseases or biomaterials for medical use (Keefer 2004 and Niemannand Kues, 2003). Therefore, engaging in goat reproductive biotechnologies appears to be profitable.

Intra Vaginal Culture is a new technique for *in vitro* fertilization, its principle consisting of fertilization of oocytes in an air-free plastic capsule, whichis placed into the maternal vagina (Sterzik *et al.*1989). *In vitro* capacitation of the spermatozoa collected from epididymis of slaughtered males used for IVF of oocytes and culture of produced embryos (Wani 2002, AL-TIMIMI 2013 and Saleh and AL-TIMIMI 2016). Many factors effect of *in vitro* fertilization like imbalance of temperature, adhesion of oocytes or embryo in early growth stage in the glass of incubation and dryness in the CO_2 incubators, and sometimes irregular the electric power, these factors represent the crucial roles for embryo and cell division and factors that effect of the oocyte quality which lead to low oocytes fertilization and low embryonic development (Kharche 2011). Oocytes play a central role in the establishment of embryonic fate. The oocyte quality

plays a major role in fertilization process and embryo development Pelletier *et al.*(2004), therefore the quality of oocytes play an important role in a proper embryo development (Marteil *et al.* 2009 and Khalil *et al.* 2005).

Intravaginal culture (IVC) is a new technique elaborated by the authors for the fertilization and culture of human oocytes. Its principle consists of fertilization and early development of the eggs in a closed, air-free milieu without the addition of CO_2. One to five oocytes are deposited in a tube completely filled with 3 ml of culture medium less than 1 hour after their recovery, with 10,000-20,000/ml of previously prepared spermatozoa. The tube is then hermetically closed and placed in the maternal vagina and held by a diaphragm for incubation for 44-50 hours (Ranoux *et al.* 1988).Stage 1 is the unicellular embryo that contains unique genetic material and is an individually specific cell that has the potential to develop into all of the subsequent stages of an animal being.

Intravaginal culture (IVC) of oocytes as a modified technique for *in vitro* fertilization. Incubated with spermatozoa (final concentration: 0.1-0.2 x 10(5)/ml) are pipette into a plastic capsule. The capsule is closed, avoiding any air inclusion. It is placed into the maternal vagina for up to 50 hours (Sterzik *et al.*1988).

Collected sperms from the tail of epididymis of the buck in the field capacitated in TCM-199 media for 30 minute only, the decrease of time may affect sperm activity [16] (Brain and Kay 1997). Oocyte recovery from mature follicle by needle 18 –G, transmit to tissue culture media TCM199 (Tulsiani*et.al* 1993).Followed by Intravaginal maturation in the eppindorf tube holding by progesterone sponges. Progesterone sponges used to protect the eppindorf tube containing the oocyte and sperm in order to decrease contraction of the vagina to fix the sponge Intravaginal (Diane 1986).The media TCM 199 used for maturation and fertilization intra vaginal .In it corresponds to the intravaginal culture of embryos and their transfer into the uterus. After being collected, the oocytes are placed, whatever their stage of maturity, in one or several 3 ml tubes completely filled with culture medium (B2 of pure Menezo) (Ranoux 1988).
Therefore, this study included: Does genitalia collected from abattoir and oocyte recovery, intra vaginal culture for maturation of oocyte, semen collected from tail of epididymis of fertile buck and Intravaginal. Fertilization of mature oocytes in the eppindorf tube by spermatozoa.

Materials and Methods

This study conducted in Baghdad University/College of Veterinary Medicine. Included collected 20 ovaries from genitalia of slaughtered does in Al-Shullaabattoir, transmit by cool container at 25^{o}C.

Oocytes recovery: Fifty oocytes were collected from mature follicles using needle gage 18, the size of follicles between 2- 3 mm bilateral side.

Oocyte maturation: by transmitted oocytes free cumulus cells to an epindroff tube containing tissue culture media (TCM-199) subsequent by intra vaginal incubation (Robertand Jeremy 2007).

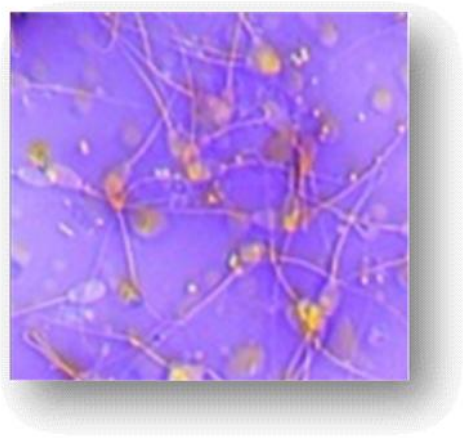
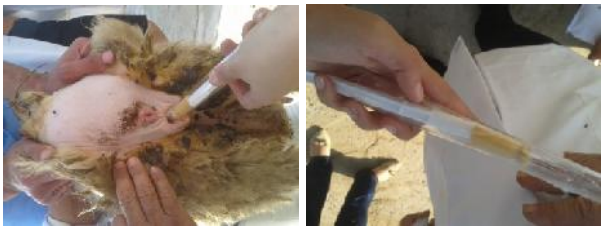


Figure 1: Buck sperms stained by eosin nigrosine.

Introduced the applicator with Progesterone sponges, which used to protect the eppindorf tube containing the oocyte (Reby *et al.* 2010).



a **b**

Figure 2 show a) Introduce the eppindorf tube in the applicator b) The applicator containing the eppindorf tube was introduced into the vagina

Semen collection: after shaving the tail of epididymis, and sterilized by iodide, spermatozoa were aspirated by syringe using needle 23-G (Roy 2004).

Intravaginal culture: (8 – 10) oocytes were mixed with 1×10^6 sperm/ml in epindroff tube containing 0.1ml of TCM199media. Eppendorf tube put in the vaginal sponge applicator and introduced into the vagina through the vulva, then incubated in the vagina. After fertilization of ovum, the media was changed (Old media was discarded and new media was added) to get rid of the sperms. Then they return to the vagina and kept until the required stage of development was reached.

Results and Discussion

Mature oocytes reached 18 out of 50 incubated oocytes, followed by incubation in the doe vagina after mixing with sperms of good motility. Every 24 hours check and investigation the contents of the tube and examined in order to evaluate the occurrence and the stage of embryonic division.

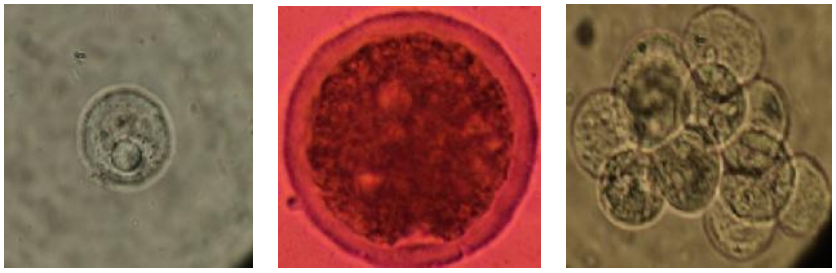
The results of microscopically examination of the oocytes in the deferent periods included 24hrs, 48hrs and 72 hrs. Gave deferent features of division: oocyte before fertilization figure 3a, pronuclear in the zygote figure 3b. A pronucleus (plural: pronuclei) is the nucleus of a sperm or an egg cell during the process of fertilization, after the sperm enters the ovum, but before they fuse, and 8-cell stages in the figure3c

The cauda epididymis large and firm used for aspirated semen. A large, firm tail is indicative of good reserves whereas a small, soft tail would indicate the opposite (Ranoux *et al.* 1988), collected semen from cauda of epididymis has been more practical for sperm count and concentration than collection by other methods (Ferriere 2005).

IVC technique minimizes exposure of gametes to factors, which may affect their early development, such as light or low temperature. Furthermore, there is an important psychological factor to be considered, as the patient actively participates in the incubation period of *in vitro* fertilization.IVC was found to be a simple, effective and comparatively inexpensive procedure compared to the conventional IVF technique (Khan *et al.* 2013).

The embryos development is resemble that developed by *in vitro* fertilization. Only one oocyte was dead, the reason of dead oocyte it may be low quality, aged, therefore oocytes fertilized and reached the deferent stages of development in growing (Krisher 2004).It is referred that Intravaginal Culture is a good technique for fertilization in the field.

In **conclusion** intravaginal culture good method for oocyte fertilization with embryo development and an easy method for fertilization in the field without CO_2 incubator.



a b c

Figure3: Show the deferent stages of oocytes and embryos. a) Oocyte before fertilization b) Pronuclear in the zygote. c) Embryo in over 8 stage.

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