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In Vitro Maturation of Goat Oocyte with Deferent Hormonal Additives and in Vitro Fertilization by Buck and Ram Sperm

Saad Akram Hatif College of Vet Med, University of Baghdad

Omar Mezher abdulla College of Vet Med, University of Baghdad

ABSTRACT

This study included the evaluation hormonal additive for tissue culture media in vitro maturation (IVM)and fertilization (IVF) of goat. With the trail hybrid fertilization of doe oocytes by ram semen. Therefore 910 oocytes collected by slicing and aspiration from goat ovary included oocytes complete surround with oocytes partial surrounded by cumulus cells , and 25 oocytes recovered for hybrid fertilization . Hormonal additive (LH ,FSH ,estrogen ,progesterone ,and total mixed) in the tissue culture media .The best result when used the mixed of total hormones additive in the TCM199 through in vitro maturation and fertilization, when the oocytes recovered by slicing or aspiration . While the progesterone used in the TCM199 gives low IVM ,and IVF in both slicing or aspiration of oocytes source .The hybrid between doe oocytes with the ram semen ,only three oocytes reached the embryonic development

KEYWORDS IVF-goat-hybrid

Introduction

The development of a technique that allows for Oocyte and early embryo manipulation is one of the major scientific endeavors in the field of genetic manipulation for animal disease model (**1**).

In vitro fertilization the egg is fertilized by sperm in a test tube : then implanted in the uterus (**2**) .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 and , 8-The presence of cumulus cells and cell growth factors in the culture media (**3**) .

In vitro fertilization , bovine serum albumin (BSA) was added to the culture medium . The reasons for this were to mimic the extra cellular milieu to prevent hormones added to the culture medium from adhering to the incubation vials (**4**)

The constant feeding regimen had a more profound effect on oocyte quality than observed shifts in the peripheral concentrations of some reproductive hormones (**5**) .

Oocytes were cultured in media with gonadotropin only, moderate (degree +2) cumulus expansion was observed in TCM-199B, while no (degree 0) cumulus expansion was observed in mWM (**6**)

Therefore this study included:1-Collection of the doe oocytes from abattoir .

2-Semen collected from epididymis of testis .3-Additive hormones (LH, FSH, Estrogen ,Progesterone , total of previous hormones)for tissue culture media TCM199 .4-In vitro maturation of oocytes .5- In vitro fertilization.

Materials and methods

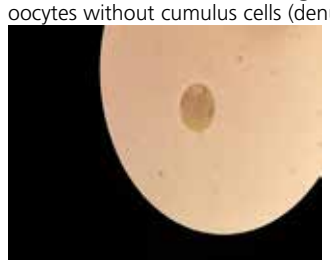
Female genital systems of the doe were collected fromAlshuala abattoir in Baghdad Province, transmitted within one hour in a normal saline at 33-35 ^{0}C in cool box, to Obstetrics lab at the College of Veterinary Medicine, Baghdad University. Oocytes recovered from follicles by slicing and aspiration **.**The oocytes were washed twice in a maturation medium either TCM-199 with deferent hormones additives (LH, FSH, Estrogen, progesterone, and total mixed hormones . They were incubated in appropriate maturation medium at 39 ^{0}C temp, 5% CO_2 and 90% relative humidity for 27 hrs. The incubated petridish was examined under inverted microscope. The presence of the first polar body was a good criteria for maturation of oocytes ***in vitro*** (IVM). The cumulus mass was mechanically removed from the oocyte by vortexing or repeated pipetting , the oocytes were washed by centrifugation and re – suspended in culture medium (**7**).

Semen were collected from the tail of epididymis by aspiration with 3ml (18- guage) The sperm then were incubated in 5% Co2, 35$_o$ c with 90% relative humidity for 4-6 hours in sterile test tubes . Capacitated sperms suspension was diluted to yield a concentration of 1.0 × 10^6 sperm/ mL in the fertilization medium (m TCM-199 with pH adjusted between 7.4-7.8. Only matured oocytes were kept in groups of 5 to 10 oocytes in petridish containing fertilization medium with spermatozoa and incubated at 39 ^{0}C, 5% CO_2, 90% relative humidity for 27 hrs (8)

Results and discussion

This study conducted of goat genitalia were collected from abattoir of Alshuala in the Baghdad province . Collected 650 oocyte by slicing and 260 by aspiration, for maturation and in vitro fertilization by epididymis buck semen ,in the total of 910 oocytes . With 25 oocyte trail for hybrid fertilization with the ram semen.

Oocytes were examined under inverted microscope and graded as in three types , the first oocytes with many layers of cumulus cells **Figure:1,a** , the second oocytes with partial surrounded of cumulus cells **Figure**:**1,b** ,and the third group oocytes without cumulus cells (denuded) **Figure:1c**



c

Figure 1: Show the deferent types of oocytes (a) many layers of cumulus cells (b) partial surrounded of cumulus cells (c) (denuded).

,the last group discarded out of IVM and IVF . This study concentrated additive the deferent hormones in the tissue culture media . Hormones should be added to the culture medium to improve culture conditions for increase the oocytes maturation rate . (**8**) Early goat embryos failed to develop to a blastocysts stage in a traditional culture media , this block occurred around time of activation of embryonic genome ,therefore serum and cells are added to the culture to avoid this block (**9**). Oocytes Maturation were collected by slicing method , in to the TCM199 with different additive **table 1** and fertilization **table 2,** the total mixed of hormones gives high percent of growing while the progesterone additive minimal effect of oocyte development .The most commonly used system for the maturation of oocytes outside the follicle is TCM199 with FSH,LH, estradiol (**10**). LH induces the expression of members of the epidermal growth factor ,this factors recapitulated LH action in vitro by promoting oocyte maturation and cumulus expansion (**11**).The mature oocytes appeared the firest poler body ,when oocytes division in 2-cells followed more developed to morella and blastocysts **Figure 2**.

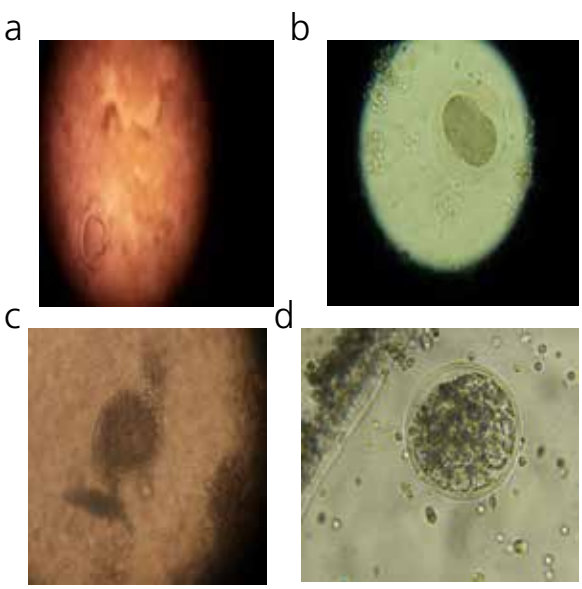


Figure 2: Show a)the maturation of oocyte with first polar body.b)2cell division ,c)modula ,and d)blastocysts .

While the Oocytes Maturation were collected by aspiration method , in to the TCM199 with different additive **table 3** and fertilization **table 4,** another the total mixed of hormones gives high percent of growing while the progesterone additive minimal effect of oocyte development.. In goat estrus goat serum are used by several workers (**12**).

The trail for maturation and fertilization of 25 oocyte doe with the semen of ram . Oocytes maturation in the TCM 199 with additive of all hormones, successfully matured and fertilization of 3 embryos ranged between 2-cell stages, and growing blastocysts stage . A male sheep impregnated a female goat in New Zealand resulting in a mixed litter of kids and a female sheep-goat hybrid with 57 chromosomes(13). In France natural mating of a doe with a ram produced a female hybrid carrying 57 chromosomes. This animal backcrossed in the veterinary college of Nantes to a ram delivered a stillborn and a living male offspring with 54 chromosomes(14).

Table 1 : Show the maturation of oocytes in the TCM199 with additive deferent hormones (Oocyte collected by slicing)

TCM199 with hormones	Oocytes number	Matured	percentage
LH	120	79	65.833%
FSH	130	82	63.076 %
ESTROGEN	140	83	59.285 %
PROGESTERON	140	86	61.428 %
MIXED HORMONE	120	82	68.333%

Table 2 : Show in vitro fertilization of oocytes in the TCM199 with additive deferent hormones (Oocyte collected by slicing)

TCM199 with hormones	Oocytes number	Cleavage	Morella	Blastocysts
LH	120	36	9	0
FSH	130	46	11	1
ESTROGEN	140	44	8	0
PROGESTERONE	140	47	10	0
MIXED HORMONE	120	44	13	2

Table 3 : Show the maturation of oocytes in the TCM199 with additive deferent hormones (Oocyte collected by aspiration)

TCM199 with hormones	Oocytes number	Matured	percentage
LH	60	39	65.0 %
FSH	40	25	62.5 %
ESTROGEN	50	29	58.0 %
PROGESTERON	50	31	62.0 %
MIXED HORMONE	60	41	68.33 %

Table 4 : Show in vitro fertilization of oocytes in the TCM199 with additive deferent hormones (Oocyte collected by aspiration)

TCM199 with hormones	Oocytes number	Cleavage	Morella	Blastocysts
LH	60	20	8	1
FSH	40	14	4	1
ESTROGEN	50	15	4	0
PROGESTERONE	50	17	4	0
MIXED HORMONE	60	23	6	2

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