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The Tissue Culture Media Supplement to Improve In Vitro Embryos Production in Ewes: A Review

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Abstract:

The Iraqi sheep production is important economically when increase interest skin, wool, milk and meats. To increase the in vitro embryo production must interest the estrous synchronization, superovulation and in vitro embryo production (IVEP) in sheep. Several successes has been in recent years in biotechnology reproduction in farm animals especially in sheep,but several limitation of IVEP in each stage procedure, so it less than ambitious. For this estimated 30-60 % from the oocyte culture reach to metaphase II in maturation stage , 20-35% from mature oocytes becoming zygote after fertilization stag and 10-25% from embryo development to blastocyst stage . Also the embryo production in vitro has low implantation rate when embryos transfers because has low quality than in vivo. For tis require development and interest of tissue culture media supplement to improve all embryo production stage and more laboratories used one or more different tissue culture supplement to improve embryo production in farm animals. The media supplement with antioxidant, hormones, fetal calf serum, follicular fluid and antibiotics, mostly all these material addition to culture media to improve embryo production in sheep. Conclusion the supplement tissue culture media to in vitro embryo production in sheep have positive effect advantage to increase maturation, fertilization and culture embryo in the laboratory experiment.

Key words: *in vitro* embryo production , supplement culture media , sheep oocyte

Introduction:

The important of sheep not only producing milk , meat , skin and wool for human right now it is considered important model to large animal for used in research in laboratory biomedical to simulator human as rat and mouse model , but sometimes it's not the same condition as which in human (Zhu *et al*., 2018).In the new year's several successes in field of reproductive biotechnology in farm animals and the small ruminant compromise a fantastic model progress in this technology , so not surprise the dolly the first farm domestic animals cloned by nuclear transfer was

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sheep (Tibary *et al*., 2005).Also the efficiency the in vitro oocyte maturation (IVM) and in vitro fertilization (IVF) in small ruminant resemble ewe and doe are more than great important compered large ruminant , due to low reproduction interval and high prolificacy (Kharche *et al*., 2009).

In the in vitro embryo production (IVEM) the 25% from oocyte used developed to blastocyst stage , this low blastocyst number attributed to the environment can not resemble the environment in vivo and produced low quality embryo with change in the morphology and decrease in gene expression , so you need to supplement the culture media to mimic female reproductive system (Gordon, 1991).For this reason the culture maturation media consider critical term of case correct environment for oocytes maturation in nuclear and cytoplasmic maturation stage because the tissue culture media supplement with different protein , antioxidant , growth factor and hormone to improve the three stage of in vitro embryo production (in vitro maturation , in vitro fertilization and in vitro culture) (Yadav *et al*., 2009).

In the most of laboratory biotechnology the tissue culture media used in vitro oocyte maturation mostly TCM 199 after supplement hormone, fetal calf serum and broad spectrum antibiotic then incubation in incubator in 5% CO2 with 39C in moistened air condition (de Souza-Fabjan *et al*., 2014). Current development in information in vitro oocyte maturation and different culture condition lead to considerable development in vitro embryo production system (Izquierdo *et al*., 19). so the review article explain role the supplement the culture media to improve all stage of in vitro embryo production in ewes .

In vitro embryo production:

In the most of important biotechnology in the domestic animals and husbandry the in vitro embryo production, nevertheless the efficacy of the production embryo laboratory is low quantity rang 25 – 45 % of oocytes arrive in to blastocyst stag and this lower result is due to the in vitro environment not mimic to the in vivo condition in female reproduction organ (Menchaca *et al*., 2018). Also the embryo different in morphology and alter the gene expression (Abd El-Aziz *et al* ., 2016). The

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IVEP technology play important role in breeding sheep in future by hasting sheep reproduction in addition to improve efficacy of this reproduction (Ledda *et al* ., 2019). Nevertheless present some many practical challenges in efficacy of IVEP in ovine such as poor embryo quality and lower in quantity , but the biotechnology system remained play vital role especially when improve genetics breeding in sheep than natural reproduction (Zhu *et al*., 2018). The advantage of IVEP, when production of offspring from infertile female, before puberty age, lactating female, pregnant female, slaughter female or dead and increasing genetic gain by shorting breeding interval (Paramio & Izquierdo, 2014).

The improve quantity and quality of blastocyst rate to attained subsequent in vitro embryo production must improve the embryo culture environment (Sutton *et al*., 2003). The tissue culture used in vitro embryo production classified according material supplement (a) when the serum supplement or somatic cell called undefined media (b) when albumin supplement called semi-defined media (c) when macromolecule supplement such as poly vinyl alcohol called defined media (Farin *et al*., 2001; Vanroose *et al*., 2001). The routine procedure to producing and development of zygote to blastocyst stage must work three consecutive stages the first stage in vitro maturation oocyte collected then fertilization by capacitation sperm and final stage culture the embryo development at the blastocyst stage (Sirard *et al*., 1988).

In vitro maturation:

The in vitro maturation stage in mammal define sequence development event occur in ova convert from germinal vesicle stage to meiotic division in second stage with formation of the first polar body (Demyda & Genero, 2011). The aims of in vitro maturation oocyte to mimic the event that happen to the immature oocyte in follicular development when resumption of the meiosis stage to be capable of fertilization (Wani, 2002). So the in vitro oocyte maturation considered as critical stage of the procedure of the in vitro embryo production and success of IVM must synchronization in nuclear and cytoplasmic maturation events (Cognié *et al*., 2004).

Different tissue culture media used in vitro maturation in small animal ruminant such as

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MEM , Ham-F10, TCM199, …ect) nevertheless, the most media widely used in laboratory the TCM199 (Paramio & Izquierdo, 2014). The TCM199 media consist of bicarbonate buffer, energy sources such as glucose, mineral, amino acid and vitamins (de Araujo *et al*., 2009). To improve the oocyte maturation rates the TCM supplement with various serum at concentration 10 - 20% as well as fetal calf serum or estrous sheep serum (Crocomo *et al*., 2016). The type of tissue culture oocyte maturation not only effect on maturation rate development to metaphase II and convert to capable in vitro fertilization with subsequent embryo development (Bavister , 1992).

In vitro fertilization:

The technique practice when culture the mature the oocyte with sperms after capacitation in the culture media in suitable time and specific condition , this technique called in vitro fertilization (Shabankareh & Zandi, 2010). The issue culture media used in IVF must capable providing sperms maturation, capacitation and sperms motility to fertilization oocyte and produced and development embryo (Kharche *et al.*, 2009). The sperms culture in media supplement by heparin to improve sperms capacitation and embryo development (Lu, & Seidel , 2004).The heparin act binding with sperms to play important role when sperm uptake the calcium during capacitation (Lane *et al*., 199). The traditional in vitro fertilization culture media synthetic oviduct follicular fluid (SOF) media after supplement fetal calf serum to heparin (Gandhi *et al*., 2000).

In vitro culture:

When the culture of zygote development 6 - 7 days after in vitro fertilization to reach blastocyst, this stage called in vitro embryo culture (Gardner *et al*., 1994). Current research attention is accompanied international to optimize and improve the tissue culture condition to increase IVF rate and the laboratory star to used new technique in dynamic culture system and microfluidic the purpose to restock media (Isachenko *et al*., 2010; Alegretti *et al*., 2011). Whenever the improvement the in vitro culture media lead to the high development competence embryos and increase blastocyst rate (Farag *et al*., 2009) . So the improving embryos culture consider key to application in IVEP and insufficiency of culture condition lead to low viability ,

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decrease development and abnormal in morphology embryo (Lonergan *et al*. 1999). In the most laboratory semi-defined media routine to embryo culture with synthetic oviduct fluid (SOF) medium supplement with fetal calf serum 10% at 5% CO2 in temperature 39C for 5- 6 days (Freitas & Melo, 2010).

Tissue culture media supplement:

The new research in biomedical laboratory to development recent complex culture media used in vitro embryo production from the simple culture media based in blood and serum (Leddaet *et al*., 2016) . Today the culture media developed and contain a high material up to 80 component include inside nutrient the vitamins and growth factors (Chronopoulou and Harper, 2015). In addition recent research result have proven the important of tissue culture condition in pre and post implantation blastocyst after embryo transfer (Nelissen *et al*., 2013) .Competition in the field of culture media has led to an increase in the standards used in clinical application, which has led to increase in option (Quinn, 2004).

In common more laboratory used TCM199 after addtion multiple supplement from different component to improve in vitro embryo production and the media mostly supplement of gonadotropin as FSH and LH or both combination to increase the maturation rate and improve viability rate to embryo (Menchaca *et al*., 2018). In the in vitro culture system in laboratory different type of culture used for in vitro blastocyst production co-culture (when cell supplement to media) , defined and semi-defined , in co- culture of sheep embryo production mostly done by TCM199 after supplement with serum and granulosa cell from oviduct (Gandolfi & Moor, 1987). Because the culture media play critical role in the maturation, fertilization and embryo development so , necessary to selection the culture media to since selection the suitable condition to improve in vitro embryo production (Gliedt *et al*., 1996). So many material objective additions to the culture media to improve in vitro embryo production such as

1- anti oxidant supplement:

The cell process metabolism produced reactive oxygen species (ROS) , and this

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material increase from two gamete when embryo production implication because cell damage (Cetica *et al.*, 2001). Sufficient conformation about the harmful ROS on oocyte in vitro maturation and in vitro embryo production (Geshi *et al.*, 2000) . The culture media supplement with thiol comment example cysteine to improve embryo production and when increase the concentration (GSH) protect the cells from oxidative stress (Cognié *et al.*, 2003). Multiple metabolic paths are reduce and prevent the oxidative damage in the embryo production, so which are by glutamine and enzyme superoxide dismutase to control the ROS level in cells (de Matos *et al.*, 2002)

The GSH synthesis during in vitro oocyte maturation play important role in other stage fertilization and embryo development, also the supplement the tissue culture media by cysteine stimulate the synthesis GSH and improve the embryo production (Iudica *et al,* (1999) . Oikawa *et al*,(2018)confirmed the benefit role GSH 150 mg/ml to improve embryo development. In country Almeeni *et al*,(2021) used the herbal extraction to antioxidant precursor or nutrient to supplement culture media in vitro maturation in sheep, when addition fenugreek extraction to improve in vitro oocyte maturation in local Iraqi ewes and other author Zalzala, *et al*,(20016) study the effect of alcoholic extraction of licorice to improve in vitro maturation in rabbit .

2- Hormone supplement:

The tissue culture media supplement the gonadotrophic hormone to improve oocytes maturation rate and total yield embryo production (Galli and Moor, 1991). Other research addition of estradiol with gonadotropin (LH and FSH) to the maturation media , the result high significant in maturation rate (Birler *et al*., 2002) .Other study confirmed the gonadotropins are primary regulation of in vitro nuclear and cytoplasmic maturation and increase important effect of gonadotropin in maturation when collected oocyte from prepubertal female (Ledda *et al* ., 1997).The FSH hormone act trigger cumulus cell expansion in vitro and this action similar mode of LH surge in vivo (Accardo *et al*., 2004). The FSH action accomplished and finished the secondary messengers such as protein kinase and cAMP (Fan *et al*., 2004).

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The progesterone other hormone supplement to the tissue culture media in dose 5mg/ml, the effect in competency of oocyte development to blastocyst and improve the embryo production (Demyda & Genero, 2011). Also reported the prostaglandin PGE2 important to resumption the meiosis stage by provoking metabolite other archidonate mutability or enhancing meiotic competence directly on oocytes (Silva and Knight, 2000). In the country Almeeni *et al* .,(2020) used the melatonin hormone to improve in vitro oocyte maturation in local Iraqi ewe. Also Shalal, M.M. (2021) confirmed the benefit effect of growth hormone in 300IU/ml to improve maturation, fertilization and embryo development in ewes. But the hormone supplement stay controversial in some studies when found significant different of oocyte maturation in metaphase II or blastocyst rate in the addition or absent hormone (O-Brian *et al*., 1994).

3- Fetal calf serum supplement:

Many type sera used in media supplement, the serum used after heated to 56C for 30 mints to inactivate the non-variable factors such as accompaniment (Puri *et al.*,2015). The function serum supplement a nutrition factors to the cell surrounding the oocytes also the oocyte herself and prevent the harding in oocyte zona pellucida when oocyte harvest from follicle environment (Cognie et al., 1991). Other study observed the high number oocyte maturation in stage metaphase II when supplement fetal calf serum from female in days 16 of estrous cycle than serum takes other days of estrous cycle or pregnant female (Murzamadiev *et al*., 1983).The fetal calf serum generally supplement the maturation media dose 10-20 % mostly after heated in 56Cfor 30 mint (Wani, 2002).

The estrous sheep serum (ESS) mostly used in ovine oocyte maturation and addition alone or with HCG to improve maturation and following stage fertilization and embryo development in juvenile sheep oocytes (Karami Shabankareh *et al*., 2011). Also when addition ESS to oocyte maturation media collected from adult ewes, the result high maturation rate (82%) when compared with fetal bovine serum (Tajik & Esfandabadi, 2003). The maturation media used in vitro embryo production in sheep supplement with 10 % serum origin from cattle (FBS ,FCS)or sheep serum (ESS)or

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bovine serum albumin to defined media pattern (Leoni *et al* ., 2006) . Synthetic serum substitute (SSS) other material to replacement fetal calf serum to improve embryo production stage in sheep (Karami Shabankareh *et al*., 2011).

All study confirmed necessary supplement the culture media by serum to improve cleavage rate and embryo development (Sagirkaya *et al*., 2004). In the male rams sperms capacitation in media supplemented with fetal calf serum , show the high fertilization rate and cleavage rate (Kątska-Książkiewicz *et al*., 2004).The serum supplement in capacitation media act to supported cholesterol efflux and this interaction consider key event in sperm capacitation (Huneau *et al*., 1994). The serea used in vitro embryo production must tested more several sample to selected the best type and concentration to improve embryo production stage (Shirazi *et al* ., 2012)

4- Follicle fluid supplement:

The follicular fluid is liquid in antrum follicle contained metabolic material to improve oocyte growth and development, so the contained reflex the oocyte quality and embryo viability (Severino *et al*., 2013). The follicle fluid contain many active ingredient such as protein , growth factors, peptide hormone , steroid , energy subunit and other undefined factors (Sutton *et al*., 2003). In sheep IVEP program, show the both heterologous and homologous follicle fluid employ to improve the oocyte maturation (Sun *et al*., 1994).So addition of human follicle fluid or sheep follicle fluid to maturation improve the in vitro oocyte maturation and fertilization , the explore the benefit effect in improve maturation belong to the growth factors , hormone and peptide contains (Cognié *et al*., 2004). The follicle fluid supplement must extract from non-atretic follicle or large follicle more than 4 mm to is being more benefit (Sunet *et al*., 1994). Almeeni *et al*,(2021) study the effect of addition different concentration of follicle fluid in Iraqi ewes to maturation rate concluded . the concentration 20% more benefit for oocyte maturation in ewe to improve the metaphase II .

5- Growth factor:

The studies confirmed the effect of epidermal growth factor (EGF) to increase

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maturation rate also in fertilization and embryos production in various species animals (Gall *et al*., 2004). In sheep when supplement media with (EGF) produced had great cumulus cell expansion and high fertilization in sheep (Grazul-Bilska *et al*., 2003). Other studies used insulin- like growth factor (IGF-I) to stimulated the oocyte maturation and increase embryo production in sheep (Guler et al., 2000). But Shabankareh and Zandi, (2010) study the synergistic effect EGF with IGF-I to supplement in different type media undefined , defined and semi defined maturation media , he conclude these hormone increase the cleavage and embryo production especially when used undefined media to improve in vitro maturation than other maturation media . When the advancement in considerate requirement of embryo development investigated, must be development of medial component and supplement according embryo need development (Thompson, 2000) . The successive culture media would similar the change in environment *development* embryo in female reproductive system, to capable the morphological and biochemical change of embryo and the physiological event in media must reflect the level of carbohydrate in female reproductive tract and decrease in stress to the embryo when development (Lane *et al*., 2003).So the better knowledge all stage physiology embryo production in vivo from pre ovulatory oocytes and development the oocytes in follicle fluid at ovulation to improve the tissue culture embryo by suitable supplement for embryo production (Vajta *et al*., 2008).

6- Antibiotics supplement:

The perfect antibiotic used for embryo production stage to improvement tissue culture media most broad spectrum activity also must be luck of toxicity to embryo , the traditional uses the penicillin in (100IU) with streptomycin (100mg/ml)has routine used in laboratory embryo production (Menchaca *et al*., 2018) . In the maturation media the antibiotic act provide prevent contaminated and growth the microorganism during oocytes culture (Uchinuno *et al*., 1996) . The increase the important of antibiotic supplement in farm animals when the ovary bring from slaughterhouse , in contrast multiple types of bacteria isolated from fluid used to ovary transport routine to the laboratory (Melander *et al*., 2018). So

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Vol. 1, No. 4, December 2023

the rate oocytes maturation and fertilization and embryo production were high significant when culture media supplement with antibiotic than absent (Paramio & Izquierdo,. 2014). Shiraziet *et al*, (2000) study the effect of penicillin- streptomycine in vitro oocyte maturation concluded , the pen-strep increase the maturation rate especially metaphase II after 24 hours culture than absent . Also many laboratory used gentamycin sulphate (50 mg/ml) to alternate to the pen-strep , the gentamycin tolerance high temperature and keep the biochemical and biological preparatory in tissue culture media , in addition some maturation system used small droplet in mineral oil in cover the surface of media to prevent contamination and temperate (Shimada et al., 2002) .

Conclusion many different supplements used to improve in vitro embryo production, some of them are used as a nutrient, and some help the oocytes and embryos to growth and proliferation, and some of them prevent bacterial contamination and prevent cell damage when act anti-oxidant used, but with that it remains the in vitro embryo production low in quantity and quality compared in vivo.

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