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Factors affecting on In-vitro Oocytes Maturation in Goats

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Abstract:

In the small ruminant like goat and sheep the efficient to in vitro maturation and fertilization technique more important compered the large ruminant because the short time pregnancy and short parturition interval and more economic value. The important and critical stage on in vitro embryo production in vitro maturation . The synchronization nuclear and cytoplasmic maturation important event in in vitro maturation oocyte in caprine embryo production . More efforts to increase the oocytes collection high quantity and quality in addition more rate oocyte maturation . Cumulus cells and homogeneity the cytoplasm important morphology category oocytes before maturation . However the multiple factors affect in the maturation in goats including methods of oocyte collection from doe , season reproduction , age the doe and physiological reproduction condition the doe during oocytes collection . Other factors related the environment condition culture of surrounded during maturation procedure. The supplement media other important factors used to increase the maturation rate such as many different material used , fetal calve serum , hormone ,follicle fluid and anti-oxidant , all these material used to increase maturation rate oocytes to mimic in vivo process in live animal . Therefore, this article was intended to address those factors that affect maturation in goats for consideration in future research in producing the largest number of mature oocyte for use in the production of embryos in goats .

Key words : in vitro oocyte maturation , goat ,media supplement

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Introduction :

Goats one animals adapted hard environment and consider as multiple production not just meats, but also milk and skin (1). Recently reproductive biotechnology used in vitro embryo production (IVP) to accelerate support of genetic characteristic in different animals in addition to being laboratory method for production embryo(2). The other advantage to IVP used to embryo production from female infertile , pre pubertal female and slaughtered female (3). In this technique procedure made up three stage sequential , the one stage oocyte collected to maturation other two stage fertilization the mature oocytes with capacitation sperm and three stage culture the zygote to blastocyst stage to transport the recipient female or storage to used later (4). In the domestic animals great progress in procedure biology reproduction technology, the caprine consider excellent Modell to use in this technique (5). The results stay low although the progress in this technique (6). The inferior results attributed to maturation stage and fertilization (7). For this reason only 30% of oocytes mature reach the blastocyst stag, researchers explain this to change embryo morphology and gene excretion however the in vitro maturation oocyte condition is a mimics the in vivo condition in live female (8). The research result showed the oocyte mature in vitro normal fertilized but doesn't have the capacity to developed when in vitro culture to blastocyst stage (9) . In goats different protocols used to embryo production however quality oocyte remains the limiting factor to reaching blastocyst stage (10). The advance in diversity and supplement in tissue culture media used on in vitro embryo production contribute to improve this result (4 , 11).This article reviews remember these factors that affect in vitro oocyte maturation in goat. In vivo oocyte maturation:

The ovary in mammals including large number from oocyte in primordial stag, some of these oocyte progress to development towards the ovulation however some of these follicle growth and other degeneration and atresia (12).In sheep and goat like other species the development sequently process for meiosis star to gremial vesical breakdown (GVBD), metaphase one and metaphase II (13) . Atresia occurs in any stage of development in mammals when the drop of estradiol from granulosa cell most important signs follicle atresia (14). During maturation oocyte long period growth and development in the growing follicle but short period between mitosis and ovulation (15) . The oocyte arrest in meiosis prophase I while in the follicle environment then development germinal vesical breakdown and stage metaphase II , all this event occur in vivo before ovulation (16). The increasing development of amount of oocyte competent in growing

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follicle it occur in late differentiation oocyte when accumulation factor (protein , Messenger RNA), this stage take part during development (17). In the end development the oocyte become capable to regulate follicle proliferation and metabolic action, these event important in regulation follicle growth (18).

In vitro oocyte maturation:

In assisted reproduction technology allowed mature oocyte in vitro by environment like in vivo maturation occur in female animals (19). The oocyte goat traditional mature in vitro grouping (10) oocytes in droplet 50 μl in temperature 39 C^o incubation 5% CO_2 to 22-28 hrs (20). In stared technique must oocytes collected from ovaries, non maturation oocyte need culture in suitable maturation media to appropriate time to prepper fertilization (21). More researches confined most species goat need 18-22hrs in the media to be ready for fertilization , in this suitable condition more oocytes (70-80%) reach metaphase II (22). The in vitro maturation oocyte divided two process nuclear with cytoplasmic stage, the resumption meiosis with advance metaphase II realize nuclear maturation also multiple cellular change must occur in oocyte to fertilize and embryo development define cytoplasmic maturation (23). So the key stage to responsible success in vitro embryo production the appropriate maturation and the defect in this stage lead to polyspermia during fertilization (24).

Factor affecting to oocytes maturation in goats:

1 – Oocytes collection method:

The collection method efficiency and category oocyte collected very important on in vitro maturation and other stage to embryo production (4). Other factor effect to oocyte the time between ovaries collection in the abattoir to the oocyte collection in laboratory , the duration 1-4 hrs detected without any harmful effect in suitable temperature (25) The result confirmed the oocyte collected from large goat follicle have more capability development to blastocyst than oocyte collected from medium and small follicle (26). The oocyte collecting in doe after slaughter from abattoir with appropriate time or from animal live after superovulation donor doe with hormone to increase oocytes collection then used laparoscope to collection oocyte or surgical method by laparotomy (27). The other source oocyte from slaughter female genital organ (ovary) the oocyte collected by three method , aspiration follicle in cortex ovary or by slicing tissue ovary and the puncture the ovary surface by suitable needle (28). Large follicle in adult doe more than 3 mm in diameter collected by aspiration but when juvenal goat the slicing and puncture method used to collection oocyte (29). Must result confined slicing and puncture produce high number of oocyte in

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ovary (4.14 , 4,22) respectively compared with aspiration (3.28) but high significant normal oocyte in aspiration than slicing and puncture technique (30). Shirazi *et al* ., 2005(31) result confirmed the aspiration technique more active to maturation oocyte collection from slaughter house doe. Other Wahid *et al*., 1992 (32) found the rate maturation with metaphase II when used aspiration , slicing and puncture without different in goat, also no significant different fertilization rate so concluded the maturation rat related to quality oocyte and media used with suitable condition culture.

2 – follicle size :

The studies confirmed the relationship between the competence oocyte and follicle size, increases competence oocyte with increase size follicle (33). Other study reveals the relationship between in vitro embryo development in goat oocyte and follicle size , so blastocyst produce from large follicle more 5mm more development and competence than small follicle less than 2mm in diameter (34). Dieleman *et al*., 2002(35) document the follicle more 3mm in size consist of more cumulus cell layer and more maturation rate , this result explore when increase the follicle size leads to increase growth oocyte meiotic competency to yield increase embryo production in vitro . Other study confirmed the development stage more important than follicle size so the more embryo yield from oocyte aspiration from 2-3 mm than 8mm this result explore the large follicle suffering atresia stage so the oocyte incomplete meiotic stage and less cytoplasmic ability (36). In both ruminate species oocyte collected from large follicle more maturation and fertilization than oocyte in small follicle (37) . In Iraq Almeeni *at al*., 2012 (38) confirmed the percentage of mature oocytes and their vitality were better in oocytes with drawn from large follicles (6-10 mm) than the small follicles (1-5 mm) .

3- Age the female doe:

The young animal used to produce embryo in vitro but the development and competence controversial (39). Because more one studies documented the embryo produce from young animals low competence than adult animal (40). When study the effect age doe quantity and quality oocyte in different type oocyte collection method, confirmed the young animals oocyte achieve same maturation rate but low in fertilization rate compared with adult doe oocyte (41). The low fertilization rate explore the insufficiency in cytoplasmic maturation result low sperm penetration to absence male pronucleus development to increase polyspermia and cleavage failure (42).This defect lead to defect in transition and expression material genetic ,so the pregnancy failure in pre and post implantation stage (43). However found individual different between oocyte same age and species animals this different related the genetic variation effect oocyte competence

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(44). Most studies advice to used gonadotropin hormone in young animals produce high oocyte collected and maturation rate equal the adult doe (45). The effect age donor in adult ewe found the high effect to oocyte development but the oocyte collected from lamb normal development to healthy embryo during the cleavage (46). But the maturation rat from lamb oocyte arrive to maturation metaphase II in rate 60% compered in 80% in adult ewe (47).

4 - Effect of season reproduction:

Goat is seasonal poly estrus breed species more hormonal change during breading season than non-breeding season in hormonal activity, this hypothesis give an explanation the effect season on IVP addition other factor the hand stress in hot climate (48). So the breeding season in doe achieves better oocyte development capability lead to more blastocyst and cleavage rat while the quality of embryo not different between different season (49) . The blastocyst rat from oocyte mature during breeding season in autumn more 50% than other season low 40% (50). Also the development competence during anestrus low rat in blastocyst and cleavage than estrus (51).

5 - Oocyte category and diameter:

The halve genetic material in embryo from female gamete but also contribute the zygote cytoplasm and protein transcription important to development embryo (52). So the essential factor in IVP the oocyte quality in embryo development before genomic activation, this activation consider the essential key for embryo development (53). The cumulus oophers cells (COCs) divided two grad according morphology categories :the number one and healthy oocyte when homogeneity cytoplasm with three layer of cumulus cell this oocyte labeling grad I but when the cumulus cell less than three layer partial cumulus cell or denuded with granulated cytoplasmic this oocyte category grad II (54). The grad I when the cumulus speculation more maturation rat when culture 24 hrs than oocyte with less three layer or denuded, so the last type rejected because low viability in fertilization and embryo development (55). Other factor the oocyte diameter who always accompanies with follicle size, the two merit increase oocyte diameter and follicle size increase the embryo production (56). Oocyte from young doe and adult same diameter produce same meiotic phase rate and the oocyte growth during development to lead 120 µm during the development ,so the oocyte less than 110 µm still in growth phase and low ability to developed after fertilization (57). The result studies remarked the critical diameter to development 110 µm associated with three layer cumulus (58).almeeni , 2017(59) study the effect of oocyte diameter in Iraqi doe , he confirmed the important diameter in oocyte maturation , this factor more relation with embryo development and the research de-

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tect the diameter 125 µm oocyte more suitable to embryo development.

6- Culture condition:

The incubation time and environment other important factor influence oocyte development , the time maturation incubation in caprine 27 hrs appear long than ovine species 24hr (60).The goats oocyte mature traditionally in TCM maturation media in 39C^{o} with CO2 5% for time 24 -27hrs (61). Other Sharma *et al*., 1996(62) document the culture doe oocyte 33 hrs in TCM media with 10 -20 % estrus goat serum the high oocyte maturation and more embryo development. The oxygen availability is important in culture oocyte during an aerobic environment so when present phosphorylation inhibiter the oocyte under arrest in germinal vesicle (63). Other result in cattle when oocyte mature during low oxygen less 10% lead to polyspermia during fertilization and low development competence (64).

7- Ovarian cycle state:

When used ovaries from slaughter house more morphology different ovary because more reproductive breed to the animal slaughter , the corpus leteum important factor affect to the quantity of oocytes when compered the ovary absent corpus luteum (65).Other the quality of oocyte high significant in ovaries absent CL when compared with bearing CL (66) In Iraq almeeni *et al*., 2020 (67) confirmed the ovaries with the corpus luteum in ewe affect to quality and quantity of oocyte without affect to maturation rate .

8- Culture media and supplement:

In embryo production technique the tissue culture media structure categorized to three type , the one when serum and somatic cell addition this media called undefined media other two type used albumin alternative to serum and three type when albumin replace in macromolecule example polyvinyl alcohol (68, 69) . Several different media used to oocyte maturation in small ruminant such as Ham F10 , MEM, TCM however TCM the media widely used in different laboratory because contained material such as mineral , bicarbonate buffer and glucose to energy source addition to vitamin and different amino acid (70) .In most laboratory TCM media supplement different compound such as hormone , follicle fluid and anti oxidant to increase oocyte maturation rate with increase oocyte viability (71) . Some laboratory preferred replace TCM with synthetic oviduct fluid media in maturation media (72).

A – Hormone:

The maturation media supplement by different hormone to increase the nuclear and cytoplasmic maturation other function increase viability of oocyte to fertilization (73) . The most hormone used the gonadotropin as presented (FSH , LH) with estradiol , these hormones high significant to oocyte maturation (74). Suggest gonado-

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tropin important role to regulation maturation but more benefit used in pre pubertal female (75). The estradiol important to improve cytoplasmic maturation by stimulation polymerase DNA with improve male pronucleus and growth factor (76). Other hormone used to increase maturation rate in ewe the melatonin , the result confirmed benefit effect melatonin to oocyte maturation in local Iraq also the concentration 10^{-6} was the optimum concentration to increase maturation rate (77).

B – Follicle fluid:

The follicle fluid collected from non atretic follicle other supplement material to improve maturation media is more effect in goat oocyte (78). The benefit the follicle fluid du to existence hormone, growth factor and different peptide (79) .when follicle fluid used to improve maturation used more 10% until appear positive effect to maturation rate compared with control (80) . also the follicle fluid from large follicle more effect to maturation than small follicle, so the supplest of maturation media with non atretic follicle fluid from more 4mm benefit effect in goat (78) and ewe (81) .

C – Serum:

The serum other more important supplement media addition to 10 -20% more effect to protect zona pellucida from harding (82) . The important serum du to the competent such as growth factor , hormone and energic material , but some competent at risk infection (83) . In caprine the serum supplement maturation media with 10 -20 % heat serum to inactivated to provide nutrition function to the cumulus cell (84) .Other searches add 10 % estrous goat serum supplement more effect than bovine fetal serum in all stage of in vitro embryo production (85) .

D - Anti oxidant:

When embryo development the oxidative stress one of limitation development by harmful effect on cell function (86) . The cumulus cell produce glutathione to reactive oxygen species inactivated only remind small amount essential to growth and development oocyte (87) . The glutathione is non protein material most important to protect the culture media from oxidative stress (88) . Other study adding herb extraction act precursor antioxidant effect to maturation media for example extract of Licorice (89) and *Tribulus terrestris* extract (67) these result improve the maturation rate and supported embryo production in country

Conclusion :

this article we dealt with most of the factors that oocyte maturation in goats, as well as the factors that affect the quantity and quality of oocytes used on in vitro maturation to be a constitution for researchers in the production of embryos, but even if the best of these factors is taken, the in vitro embryo production remains low in

quantity and quality than it in vivo (Glory be to God the Creator, create and innovate)

Reference :

1 - Souza-Fabjan JM, Locatelli Y, Duffard N, Corbin E, Touzé JL, Perreau C, Beckers JF, Freitas VJ, Mermillod P. In vitro embryo production in goats: Slaughterhouse and laparoscopic ovum pick up–derived oocytes have different kinetics and requirements regarding maturation media. Theriogenology. 2014 May 1;81(8):1021-31..

2 - Camargo LS, Viana JH, Sá WF, Ferreira AD, Ramos AD, Vale Filho VR. Factors influencing in vitro embryo production. Animal Reproduction (AR). 2018 Jul 26;3(1):19-28.

3 - Paramio MT, Izquierdo D. Current status of in vitro embryo production in sheep and goats. Reproduction in Domestic Animals. 2014 Oct;49:37-48..

4 – Cognie Y, Baril G, Poulin N, Mermillod P. Current status of embryo technologies in sheep and goat. Theriogenology. 2003 Jan 1;59(1):171-88.

5 - Tibary A, Anouassi A, Khatir H. Update on reproductive biotechnologies in small ruminants and camelids. Theriogenology. 2005 Aug 1;64(3):618-38.

6 - Pivato I. Effect of different hormonal treatments and nutrition in donnors of oocytes on 28. in vitro bovine embryo production. Universidad Federal de Pelotas. Pelotas: Brazil (Doctoral dissertation, Thesis).

7 - Blondin P, Sirard MA. Oocyte and follicular morphology as determining characteristics for developmental competence in bovine oocytes. Molecular reproduction and development. 1995 May;41(1):54-62.

8 - Abd El-Aziz AH, Mahrous UE, Kamel SZ, Sabek AA. Factors influencing in vitro production of bovine embryos: a review. Asian Journal of Animal and Veterinary Advances. 2016;11(12):737-56.

9 - Leibfried-Rutledge ML. Development potential of bovine oocytes matured in vitro or in vivo.--p. 376-383. En: Biology of Reproduction.--Vol. 36 (1987);.

10 - Paramio MT. In vivo and in vitro embryo production in goats. Small Ruminant Research. 2010 Apr 1;89(2-3):144-8.

11 - Thompson JG. In vitro culture and embryo metabolism of cattle and sheep embryos—a decade of achievement. Animal Reproduction Science. 2000 Jul 2;60:263-75.

12 - Mermillod P, Dalbiès-Tran R, Uzbekova S, Thélie A, Traverso JM, Perreau C, Papillier P, Monget P. Factors affecting oocyte quality: who is driving the follicle?. Reproduction in Domestic Animals. 2008 Jul;43:393-400.

13 - Mermillod P, Oussaid B, Cognie Y. Aspects of follicular and oocyte maturation that affect the developmental potential of embryos. JOURNAL OF REPRODUCTION AND FERTILITY-SUPPLEMENT-. 1999 Jan 1:449-60.

14 - Cahill LP, Mariana JC, Mauleon P. Total follicular populations in ewes of high and low ovulation rates. Reproduction. 1979 Jan 1;55(1):27-36.

15 – Sutton ML, Gilchrist RB, Thompson JG. Effects of in-vivo and in-vitro environments on the metabolism of the cumulus–oocyte complex and its influence on oocyte

developmental capacity. Human reproduction update. 2003 Jan 1;9(1):35-48.

16 - Mermillod P, Dalbiès-Tran R, Uzbekova S, Thélie A, Traverso JM, Perreau C, Papillier P, Monget P. Factors affecting oocyte quality: who is driving the follicle?. Reproduction in Domestic Animals. 2008 Jul;43:393-400.

17 – Wang S, Liu Y, Holyoak GR, Evans RC, Bunch TD. A protocol for in vitro maturation and fertilization of sheep oocytes. Small Ruminant Research. 1998 Jun 15;29(1):83-8.

18 - Pangas SA, Rajkovic A. Transcriptional regulation of early oogenesis: in search of masters. Human Reproduction Update. 2006 Jan 1;12(1):65-76.

19 - Abdullah RB, Wan-Khadijah WE, Rahman AN. In vitro maturation of oocytes with special reference to goat: a review. Biotechnology. 2011;10(4):351-63.

20 - de Souza-Fabjan JM, Panneau B, Duffard N, Locatelli Y, de Figueiredo JR, de Figueirêdo Freitas VJ, Mermillod P. In vitro production of small ruminant embryos: Late improvements and further research. Theriogenology. 2014 Jun 1;81(9):1149-62.

21 - Phua AC, Rahman MM, Wan Khadijah WE, Abdullah RB. Influence of in vitro maturation duration on embryo yield in goats. Malaysian Journal of Animal Science. 2015;18(2):23-8.

22 - Cognié Y, Poulin N, Locatelli Y, Mermillod P. State-of-the-art production, conservation and transfer of in-vitro-produced embryos in small ruminants. Reproduction, Fertility and Development. 2004 Jun 28;16(4):437-45.

23 - Eppig JJ. Coordination of nuclear and cytoplasmic oocyte maturation in eutherian mammals. Reproduction, Fertility and Development. 1996;8(4):485-9.

24 - Maalouf WE, Lee JH, Campbell KH. Effects of caffeine, cumulus cell removal and aging on polyspermy and embryo development on in vitro matured and fertilized ovine oocytes. Theriogenology. 2009 Apr 15;71(7):1083-92.

25 - Pugh PA, Fukui Y, Tervit HR, Thompson JG. Developmental ability of in vitro matured sheep oocytes collected during the nonbreeding season and fertilized in vitro with frozen ram semen. Theriogenology. 1991 Nov 1;36(5):771-8.

26 - Crozet N, Ahmed-Ali M, Dubos MP. Developmental competence of goat oocytes from follicles of different size categories following maturation, fertilization and culture in vitro. Reproduction. 1995 Mar 1;103(2):293-8.

27 - Kharche SD, Goel P, Kumar Jha B, Goel AK, Jindal SK. Factors influencing in-vitro embryo production efficiency of caprine oocytes: A review. Indian Journal of Animal Sciences. 2011 Apr 1;81(4):344.

28 - Berlinguer F, Leoni G, Bogliolo L, Pintus PP, Rosati I, Ledda S, Naitana S. FSH different regimes affect the developmental capacity and cryotolerance of embryos derived from oocytes collected by ovum pick-up in donor sheep. Theriogenology. 2004 May 1;61(7-8):1477-86.

29 - Wang ZG, Xu ZR, Yu SD. Effects of oocyte collection techniques and maturation media on in vitro maturation and subsequent embryo development in Boer goat. Czech

Journal of Animal Science. 2007 Jan 1;52(1):21.

30 - Hoque SM, Kabiraj SK, Khandoker MY, Mondai A, Tareq KM. Effect of collection techniques on cumulus oocyte complexes (COCs) recovery, in vitro maturation and fertilization of goat oocytes. African Journal of Biotechnology. 2011;10(45):9177-81.

31 – Shirazi A, Shams-Esfandabadi N, Hosseini SM. A comparison of two recovery methods of ovine oocytes for in vitro maturation. Small Ruminant Research. 2005 Jun 1;58(3):283-6.

32 – Wahid H, Gordon I, Sharif H, Lonergan P, Monaghan P, Gallagher M. Effect and efficiency of recovery methods for obtaining ovine follicular oocytes for in vitro procedures. Theriogenology. 1992 Jan 1;37(1):318.

33- Kharche SD, Goel P, Kumar Jha B, Goel AK, Jindal SK. Factors influencing in-vitro embryo production efficiency of caprine oocytes: A review. Indian Journal of Animal Sciences. 2011 Apr 1;81(4):344.

34 - Crozet N, Ahmed-Ali M, Dubos MP. Developmental competence of goat oocytes from follicles of different size categories following maturation, fertilization and culture in vitro. Reproduction. 1995 Mar 1;103(2):293-8.

34 - Yang X, Kubota C, Suzuki H, Taneja M, Bols PE, Presicce GA. Control of oocyte maturation in cows—biological factors. Theriogenology. 1998 Jan 15;49(2):471-82.

35 - Dieleman SJ, Hendriksen PJ, Viuff D, Thomsen PD, Hyttel P, Knijn HM, Wrenzycki C, Kruip TA, Niemann H, Gadella BM, Bevers MM. Effects of in vivo prematuration and in vivo final maturation on developmental capacity and quality of pre-implantation embryos. Theriogenology. 2002 Jan 1;57(1):5-20

36 - de Souza-Fabjan JM, Panneau B, Duffard N, Locatelli Y, de Figueiredo JR, de Figueirêdo Freitas VJ, Mermillod P. In vitro production of small ruminant embryos: Late improvements and further research. Theriogenology. 2014 Jun 1;81(9):1149-62.

37 - Lonergan P, Monaghan P, Rizos D, Boland MP, Gordon I. Effect of follicle size on bovine oocyte quality and developmental competence following maturation, fertilization, and culture in vitro. Molecular reproduction and development. 1994 Jan;37(1):48-53.

38 – AL-Maeeni IM. Study the effect of addition alcoholic extract of Tribulus terrestris on bovine oocyte maturation in vitro (IVM). Iraqi Journal of Veterinary Medicine. 2012 Dec 31;36(2):199-203.

39 - Izquierdo D, Villamediana P, Lopez-Bejar M, Paramio MT. Effect of in vitro and in vivo culture on embryonic development from prepubertal goat IVM-IVF oocytes. Theriogenology 2002;57:1431–41.

40 - Armstrong DT. Effects of maternal age on oocyte developmental competence. Theriogenology. 2001 Apr 1;55(6):1303-22..

41 - Urdaneta A, Jimenez-Macedo A-R, Izquierdo D, Paramio M-T. Supplementation with cysteamine during maturation and embryo culture on embryo development of prepubertal goat oocytes selected by the brilliant cresyl blue test. Zygote 2003;11:347–54.

42 - Velilla E, Izquierdo D, Rodriguez-Gonzalez E, Lopez-Bejar M, Vidal F, Paramio MT. Distribution of prepubertal and adult goat oocyte cortical granules during meiotic maturation and fertilization: ultrastructural and cyto-

chemical study. Mol Reprod Dev 2004;68:507–14.

43 - Tamassia M, Heyman Y, Lavergne Y, Richard C, Gelin V, Renard JP, Chastant-Maillard S. Evidence of oocyte donor cow effect over oocyte production and embryo development in vitro. REPRODUCTION-CAMBRIDGE-. 2003 Nov 1;126(5):629-37.

44 - Koeman J, Keefer CL, Baldassarre H, Downey BR. Developmental competence of prepubertal and adult goat oocytes cultured in semi-defined media following laparoscopic recovery. Theriogenology 2003;60:879–89.

45 - Kochhar HP1, Wu B, Morris LH, Buckrell BC, Pollard JW, Basrur PK, King WA. Maturation status, protein synthesis and developmental competence of oocytes derived from lambs and ewes. Reprod Domest Anim 2002;37(1):19–25.

46 - Aghaz F, Hajarian H, Shabankareh HK, Abdolmohammadi A. Effect of sericin supplementation in maturation medium on cumulus cell expansion, oocyte nuclear maturation, and subsequent embryo development in Sanjabi ewes during the breeding season. Theriogenology 2015;84(9):1631–5.

48 - Gupta A, Singh J, Anzar M. Effect of cryopreservation technique and season on the survival of in vitro produced cattle embryos. Animal reproduction science. 2016 Jan 1;164:162-8.

49 Pereira PV, Correia L, Batista R, Freitas V, Locatelli Y, Mermillod P, Souza-Fabjan J. 82 In vitro embryo production outcomes in adult goats is affected by season. Reproduction, Fertility and Development.;33(2):149-.

50 - Mara L, Sanna D, Casu S, Dattena M, Muñoz IM. Blastocyst rate of in vitro embryo production in sheep is affected by season. Zygote. 2014 Aug 1;22(3):366.

51 - Souza-Fabjan, J.M.G.; Panneau, B.; Duffard, N.; Locatelli, Y.; de Figueiredo, J.R.; Freitas, V.J.F.; Mermillod, P. In vitro production of small ruminant embryos: Late improvements and further research. *Theriogenology* 2014, *81*, 1149–1162.

52 - Schultz RM. The molecular foundations of the maternal to zygotic transition in the preimplantation embryo. Human reproduction update. 2002 Jul 1;8(4):323-31.

53 - Leibfried-Rutledge ML, Critser ES, First NL. Effects of fetal calf serum and bovine serum albumin on in vitro maturation and fertilization of bovine and hamster cumulus-oocyte complexes. Biology of reproduction. 1986 Nov 1;35(4):850-7.

54 – Sudarshan VP, Weiser T, Chintala P, Mandal S, Dutta R. Grading of Mammalian Cumulus Oocyte Complexes using Machine Learning for in Vitro Embryo Culture. arXiv preprint arXiv:1603.01739. 2016 Mar 5.

55 – Ferdous J. Collection, grading and evaluation of goat cumulus-oocyte-complexes (COCs) in view of in vitro maturation, fertilization and culture. Master of Science Thesis, Department of Animal Breeding and Genetics. Bangladesh Agricultural University, Mymensingh. 2006.

56 - Gandolfi F, Brevini TA, Cillo F, Antonini S. Cellular and molecular mechanisms regulating oocyte quality and the relevance for farm animal reproductive efficiency. Revue scientifique et technique-Office international des épizooties. 2005 Apr 1;24(1):413.

57 - Ledda S, Bogliolo L, Calvia P, Leoni G, Naitana S. Meiotic progression and devel-

opmental competence of oocytes collected from juvenile and adult ewes. Reproduction. 1997 Jan 1;109(1):73-8.

58 - Hyttel PF, Fair T, Callesen H, Greve T. Oocyte growth, capacitation and final maturation in cattle. Theriogenology. 1997 Jan 1;47(1):23-32.

59 - AL-MEENI IM. Effect of Oocyte Diameter on In VitroFertilization in Iraqi local Goats. Advances in Animal and Veterinary Sciences,June 2017 5 (6) :249

60 - Rho GJ, Hahnel AC, Betteridge KJ. Comparisons of oocyte maturation times and of three methods of sperm preparation for their effects on the production of goat embryos in vitro. Theriogenology 2001;56: 503–16

61 - Samake S, Amoah EA, Mobini S, Gazal O, Gelaye S. In vitro fertilization of goat oocytes during the nonbreeding season. Small Rumin Res 2000;35:49–54.

62 - Sharma GT, Majumdar AC, Bonde SW. Chronology of maturational events in goat oocytes cultured in vitro. Small Ruminant Research. 1996 Aug 1;22(1):25-30.

63 - Hashimoto S, Minami N, Takakura R, Imai H. Bovine immature oocytes acquire developmental competence during meiotic arrest in vitro. Biology of reproduction. 2002 Jun 1;66(6):1696-701.

64 – Boni R. Origins and effects of oocyte quality in cattle. Animal Reproduction (AR). 2018 Jul 26;9(3):333-40.

65 - Kumar NA, Paramasivan S, Sood PA, Singh MA. Micrometry of different category oocytes recovered from goat ovaries. Indian Journal of Animal Sciences (India). 2004.

66 - Islam MR, Khandoker MA, Afroz S, Rahman MG, Khan RI. Qualitative and quantitative analysis of goat ovaries, follicles and oocytes in view of in vitro production of embryos. Journal of Zhejiang University Science B. 2007 Jun;8(7):465-9.

67 - Almeeni I. M. , Hatif S. A. and Zalzala S. J . effect of corpus luteum on quantity and quality of oocytes and in vitro maturation in local iraqi ewe *Biochem. Cell. Arch.* 2020. 20(2): 5737-5740

68 - Farin PW, Crosier AE, Farin CE. Influence of in vitro systems on embryo survival and fetal development in cattle. Theriogenology. 2001 Jan 1;55(1):151-70.

69 - Vanroose G, Van Soom A, De Kruif A. From co-culture to defined medium: state of the art and practical considerations. Reproduction in Domestic Animals. 2001 Feb 3;36(1):25-8.

70 - Mermillod P, Dalbiès-Tran R, Uzbekova S, Thélie A, Traverso JM, Perreau C, Papillier P, Monget P. Factors affecting oocyte quality: who is driving the follicle?. Reproduction in Domestic Animals. 2008 Jul;43:393-400

71 - Tibary A, Anouassi A, Khatir H. Update on reproductive biotechnologies in small ruminants and camelids. Theriogenology. 2005 Aug 1;64(3):618-38.

72 - Bormann CL, Ongeri EM, Krisher RL. The effect of vitamins during maturation of caprine oocytes on subsequent developmental potential in vitro. Theriogenology. 2003 Mar 1;59(5-6):1373-80.

73 - Stubbings RB. Investigations of culture requirements for bovine oocyte maturation in vitro. Theriogenology. 1988;29:313,

74 - Widayati DT, Pangestu M. Effect of follicle-stimulating hormone on Bligon goat

oocyte maturation and embryonic development post in vitro fertilization. Veterinary World. 2020 Nov;13(11):2443.

75 - Ledda S, Bogliolo L, Leoni G, Naitana S. Follicular size affects the meiotic competence of in vitro matured prepubertal and adult oocytes in sheep. Reprod Nutr Dev 1999;39:503–8

76 MALAKAR D, Majumdar AL. Comparative study on in vitro development of goat embryos cultured in medium containing L-glutamine alone or essential and non-essential amino acids. Indian journal of dairy science. 2002;55(3):145-9.

77- Almeeni IM, Hatif SA, Zalzala SJ. study of the effect of melatonin to in vitro oocyte maturation in local iraqi ewe. plant archives, 2020 20 (2): 8891-8894

78 - Cognié Y, Poulin N, Locatelli Y, Mermillod P. State-of-the-art production, conservation and transfer of in-vitro-produced embryos in small ruminants. Reproduction, Fertility and Development. 2004 Jun 28;16(4):437-45.

79 - Zhu J, Moawad AR, Wang CY, Li HF, Ren JY, Dai YF. Advances in in vitro production of sheep embryos. International journal of veterinary science and medicine. 2018 Jan 1;6:S15-26.

80 - Algriany O, Bevers M, Schoevers E, Colenbrander B, Dieleman S. Follicle size-dependent effects of sow follicular fluid on in vitro cumulus expansion, nuclear maturation and blastocyst formation of sow cumulus oocytes complexes. Theriogenology. 2004 Nov 1;62(8):1483-97.

81 - Sun FJ, Helm P, Irvine B, Seamark RF. Effect of sheep and human follicular-fluid on the maturation of sheep oocytes in-vitro. Theriogenology 1994;41:981–8.

82 - Mogas T, Palomo MJ, Izquierdo MD, Paramio MT. Morphological events during in vitro fertilization of prepubertal goat oocytes matured in vitro. Theriogenology 1997;48:815–29.

83 - Baldassarre, H., & Karatzas, C. N. (2004). Advanced assisted reproduction technologies (ART) in goats. *Animal Reproduction Science*, *82*, 255-266.

84 - Bavister BD. Culture of preimplantation embryos: facts and artifacts. Human reproduction update. 1995 Jan 1;1(2):91-148.

85 - Wang ZG, Xu ZR, Yu SD. Effects of oocyte collection techniques and maturation media on in vitro maturation and subsequent embryo development in Boer goat. Czech Journal of Animal Science. 2007 Jan 1;52(1):21

86 - Goncalves FS, Barretto LS, Perri SH, Mingoti GZ. Bovine in vitro fertilization with cysteamine, 2 mercaptoethanol and heparin. Acta Scientiae Veterinariae. 2006;34.

87 - Tatemoto H, Ootaki K, Shigeta K, Muto N. Enhancement of developmental competence after in vitro fertilization of porcine oocytes by treatment with ascorbic acid 2-O-α-glucoside during in vitro maturation. Biology of reproduction. 2001 Dec 1;65(6):1800-6

88 - Meister A. Selective modification of glutathione metabolism. Science. 1983 Apr 29;220(4596):472-7.

89 - Zalzala S, M AL-Meeni I, H Al-Timimi I. Effect of alcoholic extract of Licorice (Glycyrrhiza glabra) on in vitro maturation of rabbits oocyte. journal of kerbala university. 2016 Mar 1;12(1):27-30.