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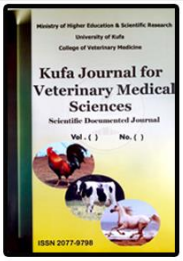

Assessment Of Differante Techniques For Oocyte Collection For Ivf In The Camel (*Camelus dromedarius)*

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Abstract:-

The aims of the present study were to compare between the harvested methods of oocytes of she camel ovaries and their effect on the numbers of oocytes collection, Grad of oocytes and ratio of maturation oocytes from AL-Najaf slaughterhouse from December 2017 to January 2018 in rutting season . The oocytes numbers, graded of oocytes and the rate of maturation oocytes. The collection of oocytes was done by aspiration, slicing and slashing .One hundred ninety quality oocytes (190) were recovered from 90 ovaries from she camel at age (4-7) years. The study revealed that slicing method yielded significantly different ($p<0.05$) number of oocytes from the others methods as 77 oocytes distribution Grad A, B and C 25 (32.4%), 30 (38.9%), 22 (28.7%), respectively. While the aspiration method yielded 54 oocytes distribution Grad A, B and C 22(40.7%), 18(33.3%), 14(25.9%), respectively. The slashing method yielded 59 oocytes distributed grad A, B and C 23(38.9%), 20(33.9%), 16(27.11%).The study appearance the aspiration method was better than the slashing and slicing method which obtained higher quality oocytes maturation percentages and significant different($p<0.05$) compared to the other methods. From the forty oocytes cultured in TCM- 199, 18 oocytes showed cumulus expansion with cytoplasmic density and first polar body, The percentages 45% (18/40 mature oocyte). The slashing method collection culture 43 oocytes 15 oocytes showed cumulus expansion and cytoplasm density and first polar body the percentage 34% (15/43). Slicing method collection in same medium, out of 55 oocytes cultured only 11 oocytes showed cumulus expansion with cytoplasm density and first polar body. The percentage was 20% (11/55). There was a significant difference ($p<0.01$) between different collected method.

Key Words: Oocyte Collection Techniques, *Camelus Dromedarius*, Fertilize Camel Mature Oocytes, Ivf

تقييم تقنيات مختلفة لجمع البيوض للتخصيب الخارجي في الجمال

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الخلاصة:

الاهداف من هذه الدراسة هو المقارنة بين طرق جمع البيوض من مبايض اناث الجمال في موسم التناسل وتأثيرها على اعداد البيوض ،وتصنيفها ،ونسبة النضوج للبيوض المجموعة من مجزرة النجف الاشرف للفترة من كانون الاول 2017 لغاية كانون الثاني 2018. جمع البيوض تم ب(السحب، التقطيع والتشطيب) . مئة وتسعون بيضة تم جمعا من تسعون مبيض من اناث يتراوح اعمارها بين (4-7) سنوات .الدراسة اظهرت ان طريقة التقطيع هي الافضل من ناحية عدد البيوض حيث اظهرت فرق معنوي بمستوى احتمال($p < 0.05$) في عدد البيوض عن الطرق الاخرى حيث جمعت 77 بيضة موزعة صنف (ا، ب ،ج) 25(32.4%) , 30(38.9%)،22(28.7%) بالتعاقب بينما طريقة السحب اظهرت جمع54 بيضة موزعة صنف(ا ، ب، ج) 22(40.7%) ، 18(33.3%) ، 14(25.9%) بالتعاقب بينما طريقة التشطيب اظهرت جمع 59 بيضة موزعة صنف(ا، ب، ج) 23(38.9%) ، 20(33.9%) ، 16(27.1%) بالتعاقب. الدراسة بينت ان طريقة السحب هي الافضل من التقطيع والتشطيب بإعطائها نسبة اكبر من البيوض الناضجة بتأثير معنوي بمستوى احتمال ($p <0.05$) مقارنة بالطرق الاخرى من 54 بيضة زرعت في وسط زرعي ($p <0.05$) 18 بيضة اظهرت توسع في خلايا القزع وزيادة كثافة السايتوبلازم وظهور الجسم القطبي الاول بنسبة 45% (18\ 40) ، طريقة التشطيب اعطت من 43 بيضة مستنبتة 15 بيضة اظهرت النضوج بنسبة 34% (15\43)بينما طريقة التقطيع انتجت 11 بيضة ناضجة بنسبة 20% (11\55) اذن هناك فرق معنوي بين الطرق المختلفة بمستوى احتمال ($p <0.05$)

Introduction:

The camel is an important livestock species uniquely adapted to hot and arid environments. The interest in developing assisted reproductive technologies and cryopreservation for the conservation of camel genetic resources has recently increased. The epididymal sperm from slaughtered or recently died animals will increase the opportunities to create semen and to establish their use for artificial insemination (AI), *in vitro* fertilization (IVF) or intracytoplasmic insemination (1,2).

This animal has many unique biological features such as low reproductive efficiency under their natural pastoral condition due to several causes such as late age of puberty (3-4 years), short breeding season, (3-5 months), long inter calving period (3 years) and along gestation period is about (13months) (3). Due to these reasons the Zoological distribution of dromedarius camel researchers studied the reproductive and genetic improvement in camel depending on manipulation of camel reproduction using modern assisted reproductive technologies (ARTs). Which include the artificial insemination, early pregnancy detection, embryo sexing, embryo transfer, cloning and in vitro fertilization (4,2)

The first dromedary (*Camelus dromedarius*) offspring was obtained from in vitro matured, in vitro fertilized and in vitro cultured abattoir derived oocytes by (5).

In vitro culture techniques have allowed the study of mammalian embryo under controlled environment condition which provides an excellent source of low cost embryo for basic research on developmental biology and physiology and for commercial application of emerging biotechnology such as nuclear transfer and trans genesis (6).

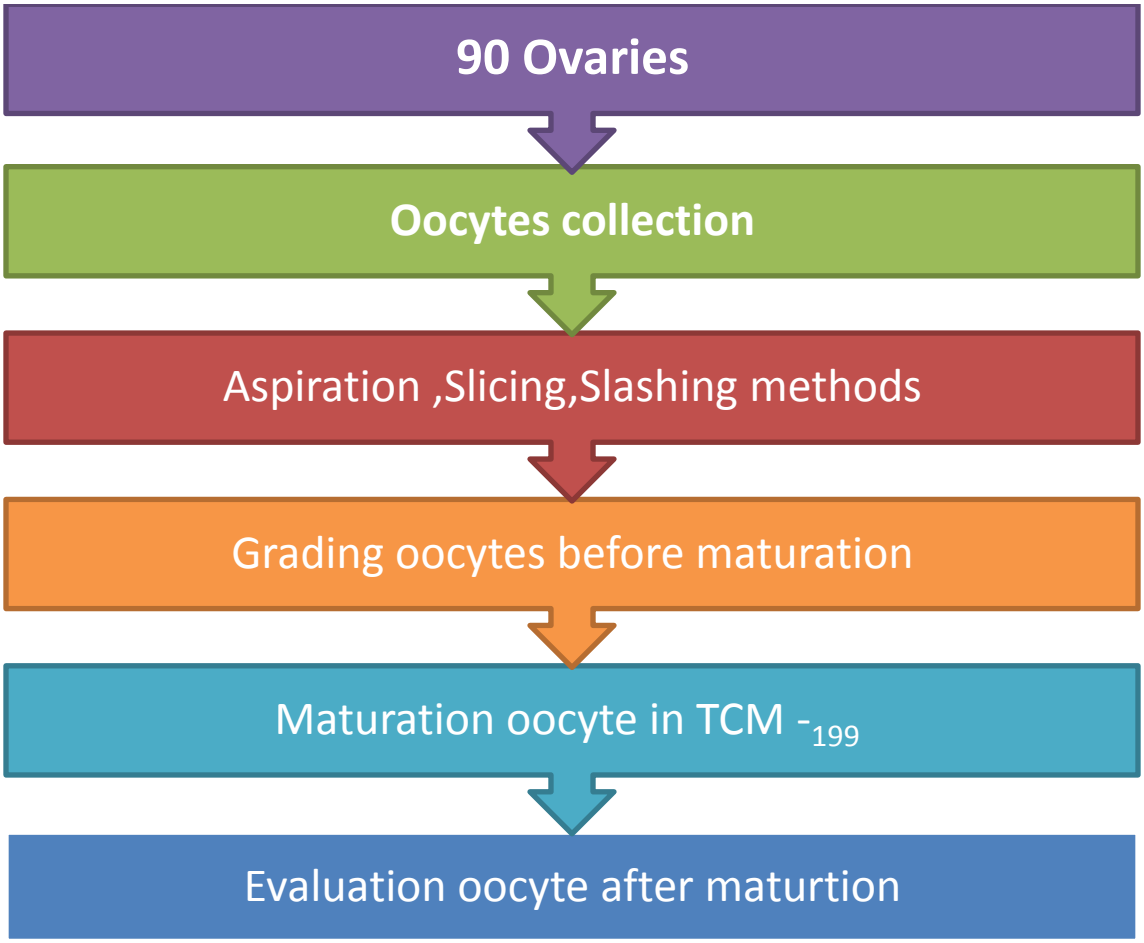
Materials And Methods:-

Samples:-

Ninty mature *Camelus dromedaries* ovaries were collected from AL-Najaf slaughter house. Then after, the ovaries were transported to the Theriogenology lab of the Faculty of Veterinary Medicine-University of Kufa by cooled box during 30 mints. The samples were collected in rutting season which began in Iraq from December (2017) to January (2018). The age of animals at four to seven years determined depends on a dental table (7).

Experimental design:-

The present graph showed the effect of different techniques for oocyte collection for IVF in *Camelus dromedarius* in TCM-199 media



Collected ovaries:-

From sexually mature *Camelus dromedarius* ninety ovaries were collected within 30 minute after murder in the slaughter house. After that the ovaries were put in physiological normal saline (0.9% NaCl) added antibiotics (100 µg/ml streptomycin sulfate and 100 IU/ml penicillin) and transported in cool boxes at 4-8 °C. They were transported through 30 mints from slaughter house to the laboratory. When reached to the laboratory all foreign tissue was removed and washed the ovaries three times in septic normal saline (0.9 % NaCl) by Ultraviolet (UV). Then, the ovaries were bath within 70% ethanol to prevent contamination and finally dried with sterilized paper towels (8).

Collected and Assessment of Follicular Oocytes:-

Oocytes were accumulated by three methods:_

1- Aspiration method:-

Thirty ovaries were used in aspiration method. Oocytes were aspirated from follicle 5 to 20 mm in diameter as (Fig1) and (Fig2) follicles using a needle gauge 20 attached to a 5 ml sterile syringe containing 2 ml media with 100 µg/ml streptomycin sulfate as

(Fig 3), 100 IU/ml penicillin and100 IU/ml Nystatin in a glass Petri-dishes (8). Oocytes were searched using a light microscope. Furthermore, oocytes were picked up with micro-pipette under light microscope and transferred into another dish containing media (9). The oocytes with intact layers of cumulus cells and homogenous cytoplasm were selected for the study (10).

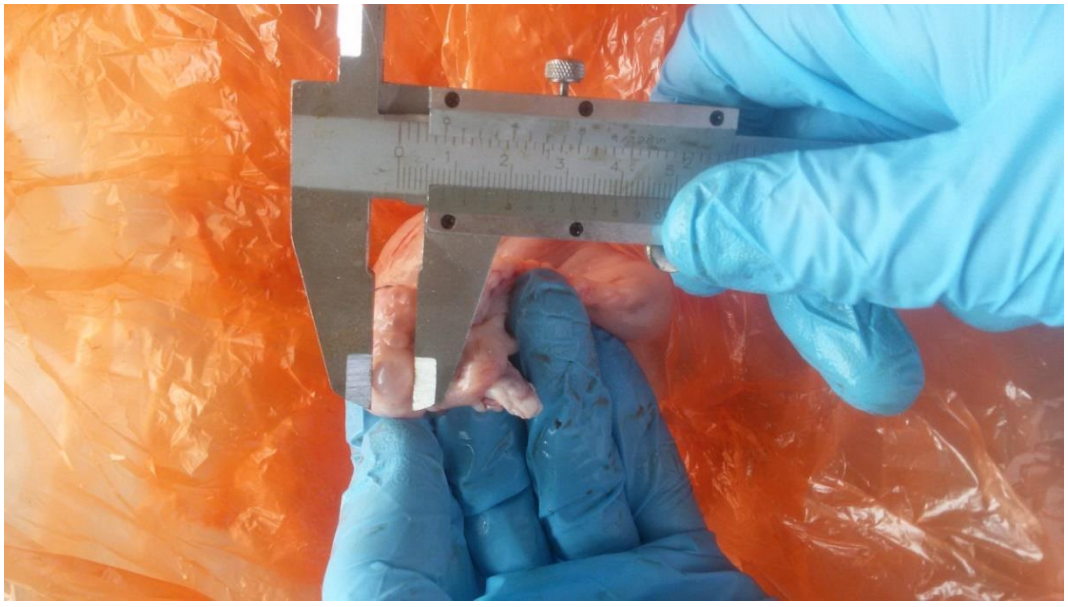


Fig 1, Ovary with follicle measurement (5 mm in diameter)

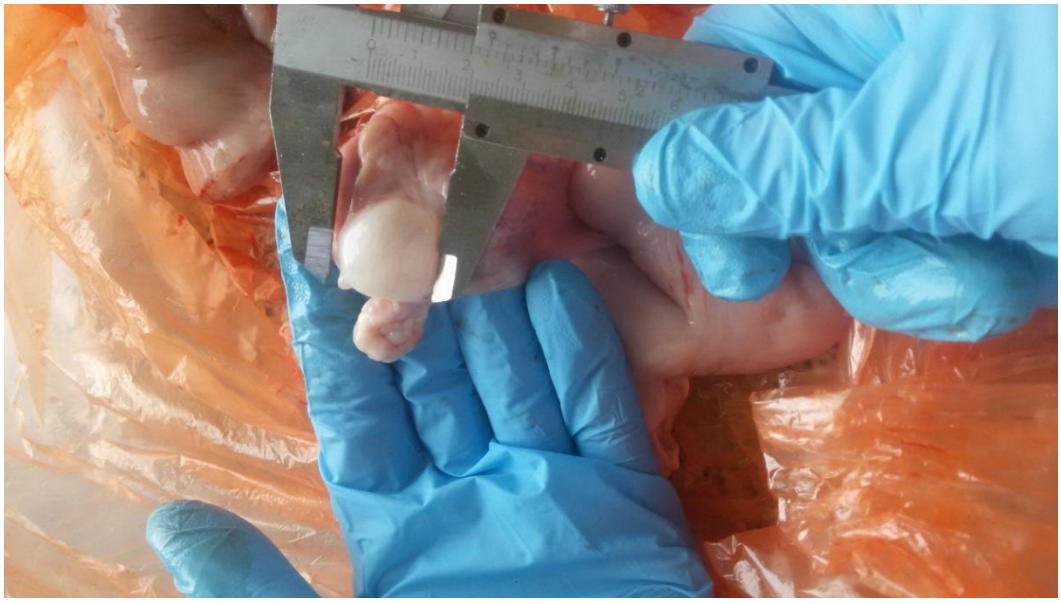


Fig 2, Ovary with large follicles (20 mm in diameter)

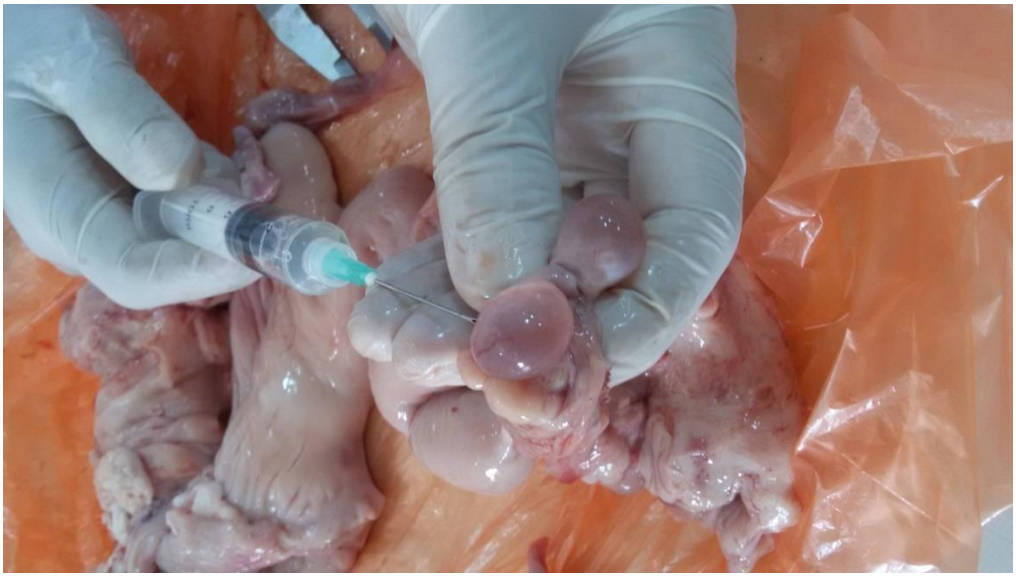


Fig 3, Follicle aspiration method for oocyte collection

2- Slicing method :-

Thirty ovaries used, the access tissue were removed (2-3) ovaries per petri dish which containing 5 mL of media with 100 µg/ml streptomycin sulfate and 100 IU/ml penicillin with100 IU/mL Nystatin, as show (fig 4). Oocytes were recovered by slicing, the surface of the ovaries slicing by sterile surgical blade. After slicing the slicing tissue was thoroughly washed in the petri dish by media were leaved to settle down at room temperature for 15 minutes. Then, the mixture examine under a light microscope to see the oocytes (11, 12).

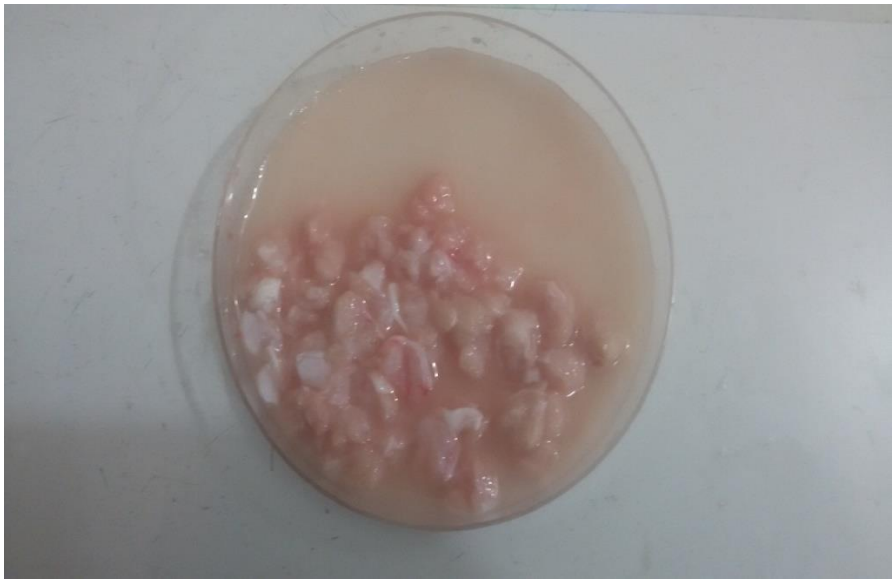


Fig 4, Show Oocytes were collected by slicing method

3- Slashing method:-

Thirty ovaries were used in this method also after collected and transported to the laboratory by cooled box (4-8)c, remove the access tissue and washing by the TCM $_{199}$ media for three time in Petri dish 60 mm, then make fife longitudinal incision in every ovaries and lotion carefully by petri dish media (Fig 5) after that leaved for serval minute then examine under light microscope and graded the oocytes then pickup grad (A,B) only to the new Petri dish with 5 ml of TCM_{199} media 100 µg/ml streptomycin sulfate, 100 I.U/ml penicillin and100 I.U/ml. Nystatin (13)

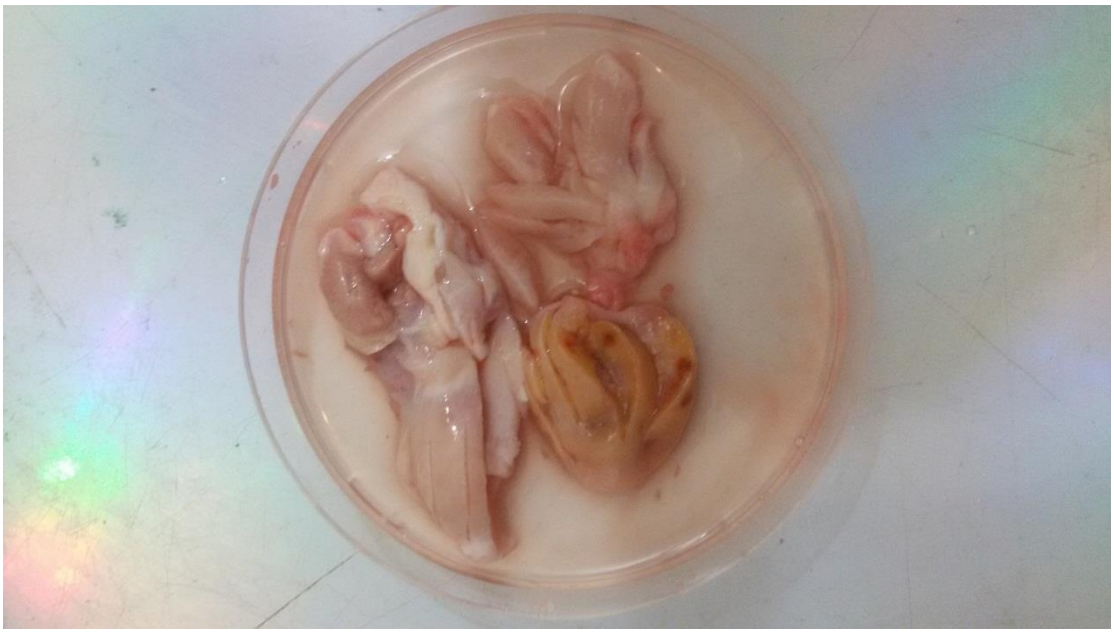


Fig 5, Showed oocytes were collected by slashing method

Oocytes Maturation (IVM):

The collected oocytes were divided for three groups depend on method of collection aspiration method GA(54 oocytes) slicing method GB (77 oocytes) slashing method GC (59 oocytes) used same Maturation medium (TCM_{199}) with Supplement 10% Bovine serum albumen (BSA), 10 IU/ml follicle stimulating hormone (FSH) was equilibrated for two hours in CO2 incubator before oocytes added, all oocytes were rinsed 2-3 times in same Maturation media with 100 IU/mL penicillin, 100 mg/mL streptomycin and 100 IU/mL Nystatin before oocytes added in Maturation medium . The culture dishes were placed in a CO2 incubator (5% CO2 at 38.5 º C, relative humidity 90%) for 30 hrs. (14). Maturation of oocytes was evaluated after 30 hrs. of culture to teste the degree of cumulus cell expansion, cytoplasm density and first polar body under a light microscope (15) the indicator for good maturation oocyte, the matured oocytes were counted and recorded. The methods described by (16). Fig (9)

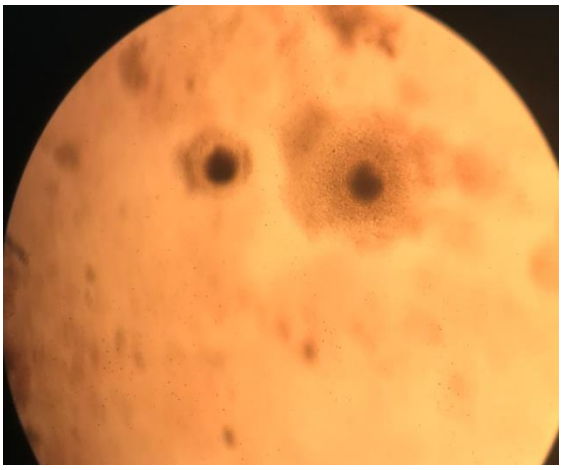


Fig 6 show Grad A oocyte

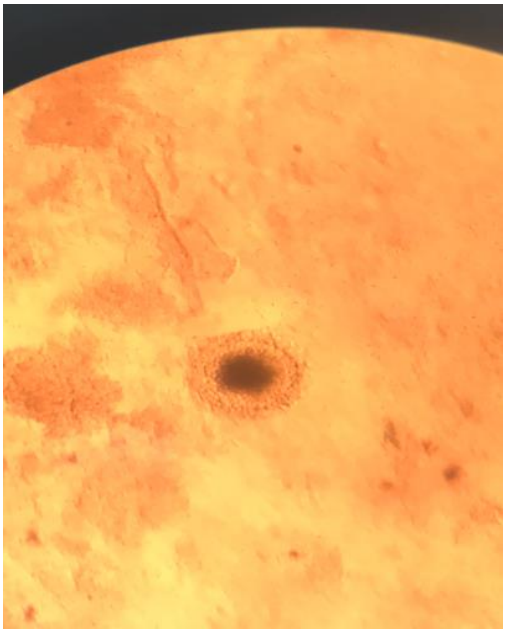


Fig 7 show Grad B 00cyte

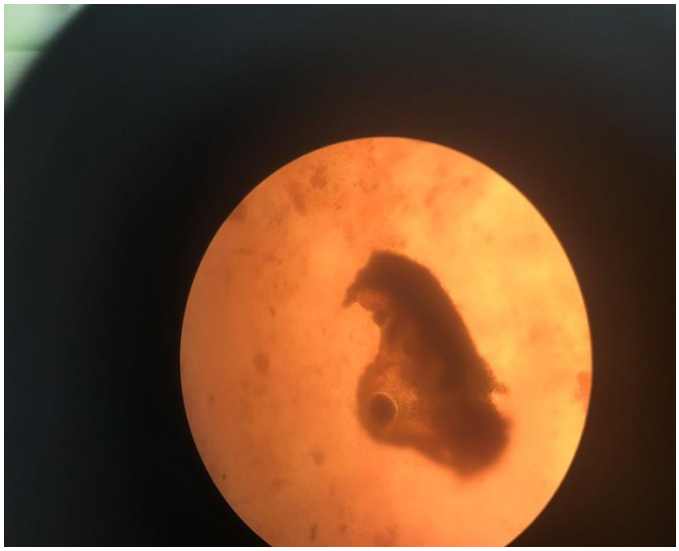


Fig 8 show Grad C oocyte

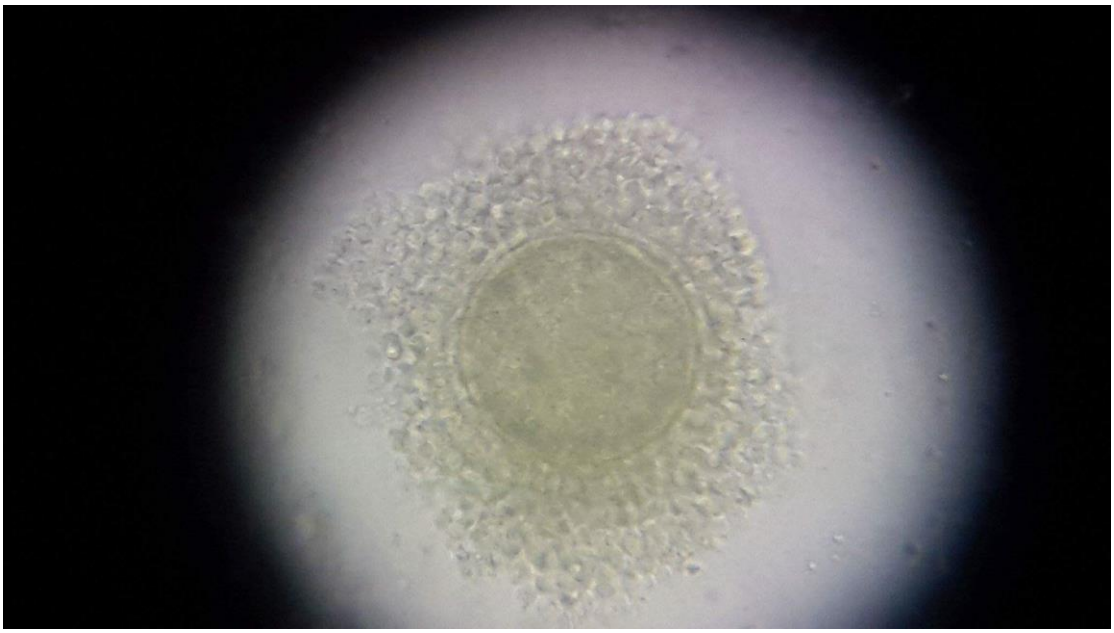


Fig 9 show maturation oocyte

Static analysis:-
Data were pooled and analyzed by *Chi-square* test using SPSS statistical software (2013).

The Effect of differences collection methods in TCM-$_{199}$ media on *Camelus dromedrius* oocyte numbers :-

The cumulus cells only The oocytes of Grades(A and B) were used for *in vitro* maturation. Data on the collection of oocytes by aspiration, slicing and slashing are presented in Table 1. In total, 190 quality oocytes were recovered from 90 ovaries from she camel at age (4-7) years in

rutting season from December 2017 to January 2018 from AL-Najaf abattoir . The study revealed that slicing method yielded higher number of oocytes 77 distribution Grad A ,B,C 25 (32.4%), 30 (38.9%), 22 (28.7%) respectively while the aspiration method 54 distribution Grad A ,B,C 22(40.7%), 18(33.3%), 14(25.9%) respectively, The slashing method 59 oocytes distributed grad A,B,C 23(38.9%), 20(33.9%),16(27.11%) respectively there was significant difference ($p<0.01$) the recovered of oocyte between three method .

Table 1. The Effect of differences collection methods in TCM-$_{199}$ media on *Camelus dromedrius* oocyte numbers.

Method	NO.of ovary	NO.OF oocyte	NO and percentages OF Grad - A- oocyte	NO and percentages OF Grad - B- oocyte	NO and percentages OF Grad - C- oocyte
Aspiration	30	b 54	22 (40.7%)	18(33.3%)	b 14(25.9%)
Slicing	30	a 77	25(32.4%)	30 (38.9%)	a 22(28.7%)
Slashing	30	b 59	23(38.9%)	20(33.9%)	b 16(27.11%)
total	90	190	70	68	52

The superscripts a, b is consider significant at ($P < 0.05$).

Effect of harvester method on maturation rate in TCM-$_{199}$ media:

The effected of collection method and percentages of *Camelus dromedarius* oocytes that showed cumulus cell expansion , cytoplasmic density and first polar body in TCM-199 are presented in Table 2.

Aspiration method 40 oocyte Only culture (grade A and B) oocytes were used for IVM and the maturation rate was assessed according to the cumulus expansion , cytoplasmic density and first polar body ,the forty oocytes cultured in TCM- 199, 18 oocytes showed cumulus expansion with cytoplasmic density and first polar body , The percentages 60% (24/40 mature oocyte) , The slashing method collection culture 43 oocytes 21 oocytes showed cumulus expansion and cytoplasm density and first polar body the percentage 48% (21/43) . Slicing method collection in same medium, out of 55 oocytes cultured only 17 oocytes showed cumulus expansion with cytoplasm density and first polar body, The percentages 30% (17/55) , There was significant difference ($p<0.05$) between different collected method.

Table 2. The Effect of differences harvester methods on *Camelus dromedarius* oocyte maturation rate in TCM-$_{199}$ media.

Method of collection	Media	NO.of oocyte cultured	NO and Percentages .of oocyte maturation rate	NO and Percentages of oocyte im maturation rate
Aspiration	TCM-$_{199}$	40	24(60%)^{a}	16(40%)
Slicing	TCM-$_{199}$	55	17(30%)^{b}	38(70 %)
Slashing	TCM-$_{199}$	43	21(48%)^{ab}	22(52%)

The superscripts a, b is consider significant at ($P < 0.05$).

Discussion:-

The collection of oocytes techniques are important in quantity and quality of oocytes recovered from the ovaries. The importance of this technique is the production of IVM-IVF embryos. This study showed the oocytes recovery rate from ovaries (slaughterhouse) was higher with slicing than the slashing and aspiration method in numbers, Similar recovery rates were observed in a studies on *Camelus dromedrius*, which is in agreement with earlier findings reporting that greater numbers of oocytes /ovary with the slicing method than with aspiration. This result was agreement with (8) reported that higher quantity oocytes were recovered in *Camelus dromedrius* by aspiration, which gave the better ratio in oocytes maturation (45%) compared with slicing method 20 % and slashing method 34% that's because we collected a good oocytes which aspiration from largest follicles that agreement with study of (17) (43%) and (18)(38.4%) and disagreement with (19) the ratio was (58.5%). (20) reported lower oocytes recovery via the aspiration method might have been because oocytes were recovered from selected follicles (5-20mm) on the ovarian surface, and follicles were limited in number (21). Mansion *Camelus dromedrius* ovaries have a more follicles (22). That mean more follicles are recruited in each cycle mansion the resulted. Slicing the ovarian surface recovered oocytes from follicles of every size, even from the follicles deep in the ovarian cortex. (20) reported that oocytes were recovered via aspiration from 55% of follicles, as

compared to the slicing method, which recovered oocytes from 78% of follicles from *Camelus dromedrius* ovaries. The superiority of the slicing method over that of aspiration for recovering oocytes in the present study is in agreement with the results obtained by (23,24). In this study the maturation oocytes appearance the first polar body that's agreement with the study of (25) .The study appearance the aspiration and slashing method gave a good maturation oocytes ratio for collected the oocytes from the ovaries of Camelus dromedaries is better than the slicing method the aspiration method ratio was (18%) then slashing method ratio was (15%) while the slicing method ratio was (11%) of maturation oocytes that's because the first two ways of collected gave better quality of oocytes and less depress and less contamination while the last method gave more than the other numbers of oocytes but the quality of oocytes were less.

References

1- Turri, F , Kandil, OM , Abdoon, AS , Sabra, H , El Atrash, A , Pizzi, F.,2013. Conservation of camel genetic resources: epididymal sperm recovery; The Camel Conference@ SOAS.. 29 April. PP: 27-32

2- El-Sayed A, Ashour G, Kamel AM, El-Bahrawy KA.,2015. Assessment of embryo production of Dromedary (Camelus dromedarius) using two semen sources and two in vitro fertilization techniques. Egypt. J. Anim. Prod. ;52:153–160.

3- Skidmore,J.A.,Billah,M.,2012.The main challenges of artificial insemination and embryo transfer in the dromedary camel. Proceeding of the ICAR Satellite on camelid a Reproduction ,3rd-5th August ,Vancouver , Canada,pp.51-56.

4- Ahmed Tibary and Abedelhak Anouassi,1996.Theriogenology in camelidae Anatomy,Physiology,Pathology and Artificial Breeding, pp447-452

5- - Khatir H.; Anouassi A.; Tibary A.,2007. Quality and developmental ability of dromedary (Camelus dromedarius) embryos obtained by IVM/IVF, in vivo matured/IVF or in vivo matured/fertilized oocytes. Reprod Domest Anim;. 42:263-270.

6- Cognie,Y.;Baril,G.;Poulin,M.and Mermillod,P.,2003 .Current status of embryo technologies in sheep and goat .Theriogenology ,59:171-188. 34-

7- -Miller, Y. and Robertson, S. .,1959. Cited by; Sisson, S. ,1975 the anatomy of the domestic animal.

8- Abdelkhalek,A.E, Sh.A. Gabr2, W.A. Khalil1, Sh.M. Shamiah3, L. Pan4, G. Qin4, M.H. Farouk., 2017 . *In vitro* production of Sudanese camel (*Camelus dromedarius*) embryos from epididymal spermatozoa and follicular oocytes of slaughtered animals Polish Journal of Veterinary Sciences Vol. 20, No. 1 . 95–101

9- Khandoker M.A.M.Y., Imai K., Takahashi T. and Hashizume . K. .,2001. Role of gelatinase on follicular atresia in the bovine ovary. *Biol. Reprod.* 65, 720-732.

10- Warriach, HM and Chohan, KR., 2004. Thickness of cumulus cell layer is a significant factor in meiotic competence of buffalo oocytes. J. Vet. Sci., 5: 247-251.

11- Mehmood, A.; Anwar, M., Andrabi, S.M.H., Afzal, M.and Naqvi, S.M.K. ,2011.*In vitro* maturation and fertilization of buffalo oocytes: the effect of recovery and maturation methods. *Turkish J.Vet. Anim. Sci*.,35:381-386.

12- Totey SM, Singh G, Taneja M, Pawshe CH., Talwar GP., 1992. In vitro maturation, fertilization and development of follicular oocytes from buffalo (*Bubalus bubalis*). J. Reprod. Fertil. 95, 597-607;

13- Saleh .W.M.,(2017) . Assessment of different methods of bovine oocytes collection, maturation and in vitro fertilization of abattoirspeciments. Iraqi jornal of veterinary Sciences,Vol.31,no.1,2017(55-65)

14- Khatir, H. ; Anouassi.,2006. A The first Dromedary (*Camelus dromedarius*) offspring obtained from in vitro matured, in vitro fertilized and in vitro cultured abattoir-derived oocytes. Theriogenology. ;65:1727–1736. [PubMed

15- El-Badry ,D.A. *et al*.,2016. **Developmental competence of Dromed-ary camel oocytes fertilized *in vitro* by frozen-thawed ejaculated and epididymal spermatozoa** Iran J Vet Res. 2016 Autumn; 17(4): 253–258.

16- Khalil, W.A .,2005 Differences between farm animals *in vitro* matured and *in vitro* fertilized. MSc Anim. Prod. Dept. Fac. Agric. Mansoura Univ. Dakahlia, Egypt.

17- Wani, N.A .;Billah M.; Skidmore JA .,2008. Studies on liquefaction and storage of ejaculated dromedary camel (*Camelus dromedarius*) semen. Anim. Reprod . Sci .109: 309-318.

18- Zeidan, AEB. ;El Hammad M.; Farouk MH.; Shamiah SM.; Ahmadi , EAA.; Nagy WMA.; Gabr SA .,2015. In-vitro maturation of camel oocytes using different media and sera. 66th Annual Meeting EAAP, 31 August – 4 September, Warsaw, Poland: Wageningen Academic Publisher, p 532.

19- Torner H. ;Heleil B. ;Alm H.; Ghoneim IM.; Srsen V.; Kanitz W. ;Tuchscherer A; Fattouh E.M .,2003. Changes in cumulus-oocyte complexes of pregnant and non-pregnant camels (*Camelus dromedarius*) during maturation *in vitro*. Theriogenology 60: 977-987.

20- Khan, I.Q., Samad, H.A., Rehman, N.U.,1997: Quantity and quality of buffalo follicular oocytes recovered by aspiration and scoring methods for in vitro studies. Pak. Vet. J; 17: 187-189

21- - Das, G.K., Jain, G.C., Solanki, V.S., Tripathi, V.N.,1996: Efficacy of various collection methods for oocyte retrieval in buffalo. Theriogenology, 46: 1403-1411.

22- Singh, R., Majumdar, A.C.,1992.: Chronological changes of buffalo follicular oocyte maturation in vitro.

Indian J. Anim. Sci., 1992;62: 205-209.

23- Sharma, D.,1990. In vitro maturation and fertilization of buffalo oocytes. M.V.Sc. Thesis. Indian Vet. Res. Inst., Izatnagr (UP),India

24- Datta, T.K., Goswami, S.L.,1998: Feasibility of harvesting oocytes from buffalo (Bubalis bubalis) ovaries by different methods. Buffalo J.; 14: 277-284.

25- Scholkamy, T. H.1; El-Badry, D. A.2 and Mahmoud, K. Gh. M .,2016. Developmental competence of Dromedary camel oocytes fertilized *in vitro* by frozen- thawed ejaculated and epididymal spermatozoa Iranian Journal of Veterinary Research, Shiraz University IJVR, Vol. 17, No. 4, Ser. No. 57, Pages 253-258.