Effect Scoring Method on Oocyte Maturation, Fertilization and Development Embryo Production from Local Buffalo Oocyte.

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Summary

The study was conducted to investigate the effect of harvesting methods (Scoring and Aspiration) on maturation oocytes, *in vitro* fertilization (IVF) and embryo development. This study was conducted at the laboratories of Theriogenology Department of Surgery and Obstetrics, College of Veterinary Medicine Basrah University, during the period extended from December 2018 to the end of March 2019. Samples from female and male reproductive system testis (50 Ovaries and 10 testes) were collected from (Al-Basrah abattoir house) after slaughter at fifteen minutes. All samples were transported in sterilize and clean boxes at (37ºC) within 1-2hrs to the center research unit. Oocytes were collected by Scoring and Aspiration methods. Only grad A and B quality oocytes were selected and incubated in an appropriate maturation medium (TCM-199) at 5% CO2, 38.5 °C and 95% humidity for 24-28 hrs. Spermatozoa were obtained by slicing of caudal epididymal of buffalo's bull. Sperms with matured oocytes were incubated in an appropriate maturation medium at 5% CO2, 38.5°C and 95% humidity from 16 -20 hrs. Fertilized ova was re-incubated in fresh media with changes 50% of media every day and examined every 24hrs for (3) days to follow embryonic development .The results showed**:** high significant (P<0.05) difference in the mean of collected oocytes in Scoring method than Aspiration method. There was high significant (P<0.05) difference in the percentage of grade A collection oocytes in aspiration method than scoring method. On the other hand the percentage of grade B and C oocytes in Scoring method was high significant (P<0.05) difference than Aspiration method. The percentages of Oocytes maturation in aspiration method was high significant (P<0.05) than scoring method, while the rate of oocyte fertilization was non- significant between two methods. Besides, the percentages of embryos development (2, 4 and 8) cells in aspiration method was significant than Scoring method.

Keywords: Reproduction, *Bubalus bubalis*, Scoring method, Aspiration method, Sperm, Maturation, Fertilization, Embrylo development.

Introduction

Buffaloes are resistant to diseases, climate and stress. It is hardy dairy animals and essential part of agricultural economy in different countries such as Iraq, Egypt, India, China, Southeast Asia, South Central America, Africa and Australia (1). Buffalo has low total number of follicles in the ovary related to low reproductive potential (2). Poor response for super ovulatory and high percentage of atretic follicles (3). Seasonality of breeding, delayed onset of reproductive maturity, silent heat, long calving intervals, low number of primordial follicles (4). many strategies had been begin to iImproving the genetic potential of water buffaloes for milk and/or meat has been a major concern for decades in many buffalo-producing countries by using biotechnology (5). In order to improve reproductive efficiency of buffalo, assisted reproductive technologies (ART), such as artificial insemination (AI), multiple ovulation and embryo transfer (MOET) and in vitro production of embryos have been introduced (6). In vitro fertilization (IVF) includes main steps that can be mentioned as the following: Collecting and maturation of oocytes, collecting and maturation of spermatozoa, fertilization and culturing (7). There are several methods of oocyte recovery, including aspiration of the follicles, dissection of the

ovaries, puncturing the follicles and Scoring have been used (5 and 8). Aims of study was
1-To investgeted number of producing embryos (obtained after incubation of collected oocyte with sperm).
2-The effect of collection oocyte methods (scoring and aspiration) on maturation, fertilization and embryos production from buffalo oocytes.

Materials and Methods

Collection of Ovaries: Ovaries of females Buffalo (3.5-7.5) years old were collected (n=50) from local Basrah slaughterhouse, collected 15 min after slaughter according to (9). And transported to the laboratory within 1 to 2 hours of slaughter, by a box containing sterile normal saline with antibiotics at 37°C. Ovaries were rinsed by 90% ethanol to reduce risk of contamination followed three rinses by normal saline to removable of ethanol traces.
Recovery of oocytes. Oocytes (n=100) were collecting by Scoring and Aspiration method:
Scoring method .Oocytes (n=65) from surface of ovaries (n=25) was collected with a sterile surgical blade, to release oocytes in a sterile 60 x 15 mm petri-dish containing TCM-199 medium (10).

Aspiration method**:**Oocytes (n=35) were collected from dominant follicles (10-20) mm use 18- gauge needle attachment to disposable syringe contained (2ml) of medium. The oocytes ware examination and classification according to their quality into three types(7).
Grade (A): Good oocytes had a homogenous granulation ooplasm and were surrounding by compacting and dense (4-6) layers of cumulus cell complexes .Grade (B): Fair surrounding oocyte by (1-3) layers of cumulus cells complexes.Grade (C): Denuded Oocytes had irregular Ooplasm and were completely empty of cumulus cells complexes layers around zona pellucid.

Experiment 1:Oocytes Maturation (IVM): Fifty (50) ml of maturation medium with Supplement was equilibrated for 1hrs. in CO_2 incubator before adding oocytes (11). 100 oocytes were recovery by scoring and aspiration methods, all recovered oocytes were rinsed 2- 3 times in the same maturation media with the addition of 100 IU/ml penicillin streptomycin and 100 IU/ml Nystatin. Culture dishes containing oocytes were putting in a CO_2 incubator environmental at (38.5ºC, 95% relative humidity and 5% CO_2) for 20- 28 hrs. Maturation oocyte was evaluated by appearance of first polar body and expansion cumulus cells to excellent as described by (12).

Experiment 2: Spermatozoa Collected, Maturation and Capacitation: collected samples (10 testis with completed attached epididymis) the organ was washed by distill water firstly, then with normal saline containing antibiotics and anti-fungal .Tunica vaginitis was removed by using a small sterile scissor, separation of the epididymis from the testicle was performed. It was cut from the whole epididymis and put in glass Petri dishes. It was Injected 3 ml of the media either TCM-199 containing 100 IU/ml penicillin streptomycin and100 IU/mL of Nystatin using different sizes gauge needle attached to a 5-ml syringe (13) . It was sliced by sterile blade for small pieces (14). After collection of sperm from caudal epididymis,it was put in 10ml test tube containing 2ml maturation media (TCM-199) with Nystatin and Penicillin , Test tube was incubated at 37 ºC for 4 hrs , the loose of cytoplasmic droplet from spermatozoa was the criteria of sperm maturation (13). To complete the next step (sperm capacitation), 50 IU/ml heparin were added at the last 45minutes of the time of maturation to complete the step of sperm maturation and sperm Capacitation (14).

Experiment 3:-In vitro fertilization:Ten matured oocytes were washed twice with medium supplied with antibiotics and antifungal before transferred to a glass Petri-dishes containing IVF medium which is supplemented with 10% FSH, 10% FCS and10%LH (15). Capacitated spermatozoa sample was prepared and diluted to yield 1-2×10^6 sperms needed for fertilization (18). After that, Petri- dishes which containing mixture Gametes was incubated at (38.5ºC, 95% humidity and 5% CO2) for 14- 18 hrs (16 - 18). Every 24h investigated to 3 days was check to sure and recorded the occurrence of fertilization. The cleavage rate of embryo was estimated according to following equation:

$$\textbf{Cleavage rate of emb.} = \frac{\textbf{Total number of cleaved oocyte}}{\textbf{Total number of cultured oocyte}} \times 100$$

(18).

Statistical analysis: Chi-square test was use for comparison between maturation and cleavage rate of embyreo fertilization rates among different groups (19). A significance level was used(5%).

Results and Discussion

Collection of oocytes Techniques : Total ova collected by two methods were (100). Scoring method increased significantly value (P< 0.05) than aspiration method. Data on the collection of COCs by scoring and aspiration are presented in (Table, 1). The study revealed that Scoring method yielded significantly higher of 2.6±0.35 (65/25) oocytes/ovary than aspiration method 1.4±0.25 (35/ 25)oocytes/ ovary .

Table,1: The recovery method on quantity of buffalo oocytes.

Methods	Ovary (No.)	Oocytes collection (No.)	Oocyte / ovary (M±SE)
Scoring Method	25	65 A	(2.6±0.35) a
Aspiration Method	25	35 B	(1.4±0.25) b

*Different small letters vertically denote significant (P<0.05) between methods oocyte collection.

Effect of the harvest methods on the quality oocytes collected from buffalo. : Aspiration method yielded significantly higher percentage of Grade A oocytes collection than Scoring method 68.5% (24.5/35), 27.6% (18/65) respectively(Table, 2). While the percentage Grade B,C oocytes collection in Scoring method significantly value than Aspiration method 23.0% (15/65), 49.2%(32/65), 22.8% (8/35), 8.5% (3/35) respectively. There was significant difference (p<0.05) in the recovered grading oocyte between two method

Effect of the harvested methods on the oocytes maturation: Data of the present study revealed increased in maturation of grade A and B oocytes collected by aspiration method compared with Scoring method . It was found that the percentage oocytes maturation was significantly (P<0.05) in Aspiration method as compared with Scoring method [81.2% (26/32) 33.3% (11/33)]respectively as (Fig. 1 and Table, 3).

Effect of Collection Methods on Embryo development-: The percentage of divided (2), (4), (8) cells embryos at 24, 48 ,72 hrs. in aspiration method a significantly (P<0.05) than the percentage of divided (2), (4), (8) cells embryos at 24,48,72 hrs in scoring method 44.4% (8/18) , 27.7% (5/18) , 16.6% (3/18) , 16.6%(1/6), 16.6%(1/6) 0.0%(0/6) respectively as (Fig. 3-5 and Table 4 and 5).

Table, 2: Effect of the harvest recovery methods on the quality of oocytes collected from buffalo.

Methods	oocytes collection (NO.)	Grade (A)oocytes collection (NO. and %)	Grade (B)oocytes collection (NO. and %	Grade (c)oocytes collection (NO. and %)
Scoring Method	65	18 (27.6%) B	15 (23.0%) A	32 (49.2%) A
Aspiration Method	35	24 (68.5%) A	8 (22.8%) b	3 (8.5%) B

*Different small letters vertically denote significant (P<0.05) between methods of grading oocyte collection (X=19.798).

Table, 3: Effect of methods collection on In vitro maturation (IVM) of grading A and B oocytes.

Methods	Oocytes culture (No.)	Oocytes maturation (No.)	Oocytes maturation (%.)
Scoring Method	33	11 b	(33.3%) B
Aspiration Method	32	26 a	(81.2%) A

*Different small letters vertically denote significant (P<0.05) between methods of oocyte maturation(X=15.212).

Table, 4: Effect of methods collection on In vitro fertilization (IVF) of oocytes maturation in different methods.

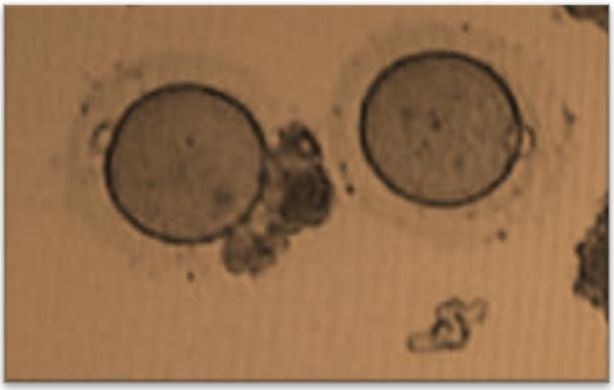
Methods	NO. of oocytes insemination	NO. of oocytes fertilization	percentage of oocytes fertilization
Scoring Method	11	6	(54.5%) A
Aspiration Method	26	18	(69.2%) A

*Different small letters vertically denote significant (P>0.05) between methods of oocyte fertilization(X=0. 731).

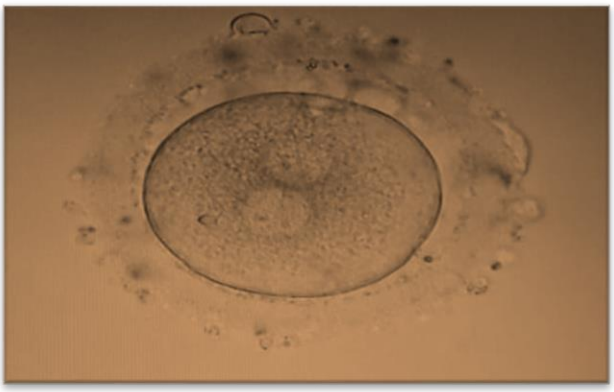
Table, 5: Effect of methods collection on Embryo development culture (IVC) of oocytes fertilization in different methods .

Methods	Oocytes fertilization (NO.)	2cells embryos at 24hrs. (NO and %)	4cells embryos at 48hrs. (NO and %)	8cells embryos at 72hrs. (NO and %)
Scoring Method	6	1 (16.6%) b	1 (16.6%) b	0 (0.00%) B
Aspiration Method	18	8 (44.4%) a	5 (27.7%) a	3 (16.6%) A

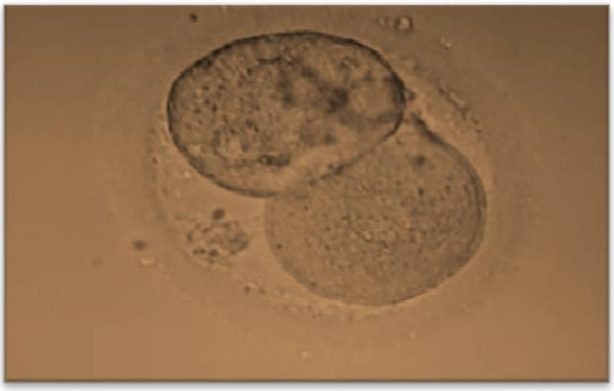
*Different small letters vertically denote significant (P<0.05) between collection methods of embryo development (X=15.212).



Figure,1: Maturated Oocyte (10x).

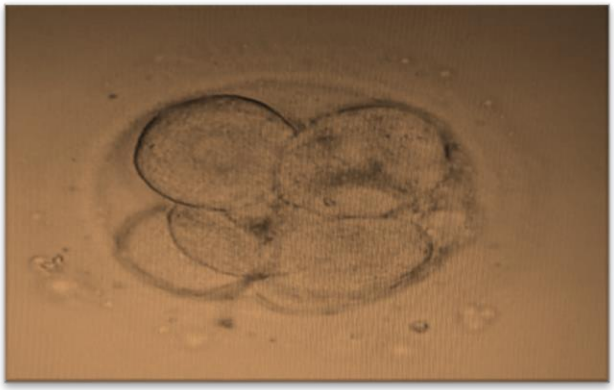


Figure,2: Fertilized Oocyte (10x)



Figure,3: 2 cells Embryo (10x)

Figure,4: 4 cells Embryo (10x)



Figure,5: 8 cells Embryo (10x)

Quality and quantity of oocytes collection per ovary are essential concerns in the production of IVM-IVF embryos. Collection oocytes and their grading in the lab are important for production of successful embryos. On the other hand appearing of a competed of cumulus cells intact a rounding the oocytes and homogeneous appearing ooplasm have been considering the superlative criteria for the selection of oocytes most likely to undergo maturation and embryonic development. The ability of buffalo oocytes is affected by a broad control of biological (20) and environmental (21) factors. Some influencing factors, i.e. size ovarian, collection oocytes methods, season, and stages of ovarian cycle on quality and quantity of buffalo follicular oocytes. The results showed that scoring method has higher number and morphologically good quality of grading (B and C) oocytes than aspiration method (10) also describe that best quality oocytes were collected per ovary in buffalo by scoring (2.6) than by aspiration (0.9) methods. In sheep, goat and cows, the quantity of oocytes collected per ovary was higher by scoring than aspiration method.

The collection rates of ovarian follicular oocytes appear in the present study are higher than those reported by (22) who obtained 0.46 usable oocytes per ovary using the aspiration method also (23) who reported comparatively low collection of follicular oocytes in buffaloes than cows might be due to the lower number of primordial and graffian follicular population in the buffalo ovaries . Cumulus or somatic cells surround the oocytes is necessary to assist the transported of nutrients and signals into, and out of the oocyte (24). The

cumulus cells rounding the oocytes play a supporting role by assisting the entry of important products and sending instructive signals to the oocyte through the gap junction for maturation (24 and 25). Oocytes surrounded by tight and multilayered cumulus investment containing ooplasm with homogeneous appearance are most likely to be developmentally competent. Oocytes that do not possess these characters complete meiosis at a lower frequency in vitro (26).

Mohammed and his colleague (27) mentioned that the percentage of oocytes maturation recovered by aspiration method (86.4%) was better than the percentage of oocytes maturation recovered by scoring and slicing method (63.3% vs…..%??) It is possible that the scoring may release less developmentally competent oocytes from follicles deep in the cortex. (28) It has been shown that oocytes released from embedded follicles in the ovarian cortex via scoring are not as meiotically competent as those from similarly sized follicles located on the bovine ovarian surface(28). Also (29), was recorded that the percentage of oocytes maturation recovered by slicing or scoring method (58.68%) the reason of low percentage of oocyte maturation by slicing or scoring method due to damage in cumulus cell and contamination during oocytes collection. On other hand (30 and 31) recorded that the damage in cumulus cells surrounding the oocyte , which plays an important role on maturation oocyte and represented by production the metabolic materials by cumulus cells that affect the efficiency and development of the oocyte.

It can be concluded that the percentage of oocytes maturation and embryos development in Aspiration method was better than Scoring method .

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تأثير طريقة التشطيب على انضاج البيوض و اخصابها وتطورانتاج اجنة الجاموس العراقي المحلي

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الخلاصة

هدفت الدراسة تأثير طرق الجمع على نسبة انضاج واخصاب وانتاج اجنة الجاموس المحلي. أجريت الدراسه في مختبرات كليه الطب البيطري فرع الجراحه والتوليد جامعه البصرة للفتره من شهر كانون الثاني 2018 لنهاية شهر اذار 2019 جمعت نماذج الدراسه الاجهزه التناسليه الانثويه للجاموس وخصى ذكور الجاموس (عدد المبايض التي تم الحصول عليها 50 مبيض وعدد الخص 10خصيه) بعد الذبح مباشرة بخمسة عشر دقيقة من مجزره البصره العصريه. ، حيث نقلت نماذج الدراسه في حاويات خاصه نضيفه ومعقمه تحتوي على محلول فسلجي وبدرجه حرارة(37) درجه مئويه خلال مدة (1-2) ساعة الى وحدة الابحاث المركزيه . تم أستحصال البيوض من المبايض بطريقة التشطيب Scoringوطريقةالسحب Aspiration . أختيرت البيوض من صنف A,B فقط لغرض الأنضاج . حضنت البيوض المختارة في الوسط الزرعي TCM-199 في حاضنه ثنائي اوكسيد الكاربون بنسبه 5% والرطوبه النسبية 95% وبدرجة حراره 538. درجه مئويه لمدة 24-28 ساعة. اما بالنسبه للحصول على النطف عن طريق اسئصال ذيل البربخ للثيران. حضنت البيوض والنطف الناضجه في الوسط الزرعي في حاضنه ثنائي اوكسيد الكاربون بنسبه 5% والرطوبه النسبية 95% وبدرجة حراره 538. درجه مئويه لمدة 16-20 ساعة . تم عزل البيوض المخصبه واعادتها الى الوسط الزرعي ، تم متابعه تطور الاجنه كل 24 ساعه مع تغير 50% من حجم الوسط الزرعي يوميا بوسط زرعي طازج ولغاية (3) أيام لغاية تطوير الاجنه ، وقد اظهرت النتائج التاليه.

وجود فرق معنوي في معدل عدد البيوض التي جمعت بطريقة التشطيب ($P<0.05$) مقارنتا بطريقة السحب، كذلك بينت الدراسة وجود فرق معنوي في نسبة عدد البيوض نوع A التي جمعت بطريقة السحب ($P<0.05$) مقارنتة بطريقة التشطيب من ناحيه اخرى بينت الدراسة وجود فرق معنوي في نسبة عدد البيوضC (B،) التي جمعت بطريقة التشطيب مقارنتة بطريقة السحب . كما لاحظ من نتائج الدراسة وجود فرق معنوي ($P<0.05$) في نسبة عدد البيوض الناضجه بطريقة السحب مقارنته مع طريقة التشطيب ، كذلك وجد من خلال نتائج الدراسة لايوجد فرق معنوي ($P>0.05$) في نسبة عدد البيوض المخصبه في طريقة السحب مقارنه بطريقة التشطيب ، كما تبين من الدراسه وجود فرق معنوي ($P<0.05$) في نسبة تطور الاجنه بطريقة السحب مقارنه بطريقة التشطيب.

الكلمات المفتاحية: التناسل ، جاموس المستنقعات ، طريقه التشطيب، طريقه السحب ، الانضاج ، الاخصاب ، تطور الاجنه .