Research Article

AL- ANBAR JOURNAL OF VETERINARY SCIENCES

Vol. 14 Issue:1, (2021) ISSN: P-1999:6527 E-2707:0603

Effect of Vitamin C on In *Vitro* Maturation of Iraqi She-Camel Oocytes

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Doi: https://doi.org/10.37940/AJVS.2021.14.1.8

Received: 12/11/2020 Revised:11/5/2021 Accepted:27/5/2021

Abstract

This study aimed to know the effect of vitamin C on in vitro oocytes maturation of Iraqi she-camel with different techniques collection. Several oocytes collection technique have been used: Three hundred ninety oocytes were collected from 84 ovaries from Afak slaughterhouse within half to one hour of slaughter animal and transport by cool box contain normal saline 0.9% (20-25C°) to laboratory of Al-Diwaniyah veterinary Hospital within 1-2 hours. After washing the ovaries with normal saline, each 28 ovaries: oocytes collected by one of the following techniques: Aspiration, slicing, and dissection. Oocytes collected from the three techniques counted and graded. Only grades A and B were selected, and undergo in maturation process. and matured in maturation medium (M199-A) and incubated in CO2 incubator at 5% CO2, 38.5 Cº, and 90% humidity for 24h and supplement the media with 0, 25, 50, 100 µg/ml of vit.C for each technique. Aspirated oocytes were cultured the results was expended cumulus cells was 66.66% (66/99) and the appearance first polar body (F.P.B.) was 68.1% (45/66). Maturation medium supplement with 50µg/ml of vit. C higher rate (84% & 81.8 expended cumulus and F.P.B.) than other groups with significant (P<5%). Maturation oocytes by slicing technique were cultured, expended cumulus was 61.4% (59/96) and the appearance F.P.B. 62.7% (37/59). Maturation medium supplement with 50µg/ml of vit. C (79.16 & 73.68% expended cumulus and F.P.B.) higher rate than other groups with significant (P<5%). Maturation oocytes by dissection technique were cultured. expended cumulus was 57.2% (59/103) and the appearance F.P.B. 55.93% (33/59). Maturation medium supplement with 50µg/ml of vit. C higher rate than other groups with significant (P<5%).

Keywards: Vit.C , IVM , She-camel Oocyte , Iraq

تأثير فيتامين ج على انضاج بويضات اناث الجمل العراقية

الخلاصة

الهدف من الدراسة الحالية هو معرفة تأثير فيتامين ج على اضاج بويضات الإبل العراقية مختبريا بتقنيات الجمع المختلفة.استخدمت عدة تقنيات لجمع البيوض. 390 بويضة من 84 مبيض جمعت من مجزرة عفك بعد ذبح الحيوان بنصف الى ساعة. نقلت المبايض بواسطة صندوق مبرد يحتوي على محلول فسلجي0.9% بدرجة حرارة (20-25م) مغذى بمضاد حيوي ومضاد فطري ونقلت الى مختبر مستشفى البيطري في الديوانية. كل 28 مبيض جمعت البويضات بإحدى التقنيات التالية: السحب والتقطيع والتشريح. البويضات التي جمعت بالطرق الثلاث عدت وصنفت. فقط نوع أ وب اختيرت للإنضاج ضجت بوسط زرعي وحضنت بحاضنة ثاني أوكسيد الكاربون 5% بدرجة الحرارة 38.5م ورطوبة 90% لمدة 24 ساعة وتم إضافة 0, 25, 50, 100 فيتامين ج للوسط الزرعي. البويضات جمعت بواسطة تقنية السحب أعطت نتائج توسع خلايا القزع بمعدل 66.66% (66/99) وظهور الجسم القطبي بمعدل 68.1% (66/45). الوسط الزرعي المغذى ب 50 مغم/مل فيتامين ج أعطت اعلى معدل إنضاج (84% توسع خلايا القزع و81.8% ظهور الجسم القطبي) بمستوى معنوية 5%. نضجت البويضات المجمعة بتقنية التقطيع كان معدل توسع خلايا القزع 61.4% (96/59) وظهور الجسم القطبي 62.7% (59/37). الوسط الزرعي المغذى ب 50 مغم/مل فيتامين ج أعطت اعلى معدل إنضاج (79.16% و73.68% توسع خلايا القزع وظهور الجسم القطبي على التوالي) عن باقي المجاميع بمستوى معنوية 5%. نضجت البويضات المجمعة بتقنية التشريح كان معدل توسع خلايا القزع 57.2% (103/59) وظهور الجسم القطبي 55.93% (59/33). الوسط الزرعي المغذى ب 50 مغم/مل فيتامين ج أعطت اعلى معدل إنضاج عن باقي المجاميع بمستوى معنوية 5%.

Introduction

Camels considered neglected animals due to the lack of studies on reproduction (1). Despite its importance in desert areas because it is an important source for the production of milk and meat, as it covers about 7% of the meat and 3% of the milk from the global product (2-4). Camel racing was popular in the early 1990s, which is a profitable and commercial resource as well as a recreational sport in the Arabian Gulf (5, 6).

Camels considered under the influence of natural conditions with low reproduction for several reasons, including the length of pregnancy, about 13 months, and lactation may last for 10 months, which leads to a prolonged period of anestrus. Therefore, it was necessary to introduce assistive reproductive technologies to understand the functions of reproduction, to increase the offspring of the species, and to obtain the best genes (7, 8). After the success of the embryo production process for Arabian camels, it led to overcoming many obstacles resulting from infertility, such as deformities of the oviduct and lack of embryo collection after super ovulation (9). In addition to that, in vitro fertilization of Arabian camels contributed to understanding the mechanisms of fertilization and early embryonic development and opening the horizons for other techniques such as cryopreservation of oocytes, embryo cryopreservation, embryo sexing, genetic transit, and cloning (10).

In vitro maturation (IVM) of oocytes face obstacle oxidant factor has a negative role in embryo development (11-14). While in another side several studies measured the effect of antioxidant on in vitro maturation and early development of embryos (13, 15-17).

One of these antioxidants is vitamin C (Ascorbic acid) which is a water-soluble material found in ovaries, corpus luteum, and follicle fluid (18, 19). It's also has another role by reducing the follicle apoptosis (20, 21), and reactive oxygen species intracellular (22).

There is no study available work about effect of vitamin C on IVM in Camel in Iraq. The aimed of this study to establish the effect of vit.C on oocytes maturation of Iraqi she-camel.

Materials and Methods

M199-A supplement with Earle's Salts, and L-Glutamine , antibiotic/ antifungal (composition for each ml: penicillin G 10000 IU, streptomycin sulfate 10mg and 0.025 mg of Amphotericin), Fetal Calf Serum (FCS) from Capricorn scientific Germany , BPS from bio-world scientific Germany, Pregnant Mare Serum Gonadotrophin (PMSG, or eCG) from Syntex Company Argentina, and vitamin C from Riedel-dehaen Germany.

Ovaries of camels collected from Afak slaughterhouse and transport by cool box contain 0.9% Normal saline (20-25Cº) supplement with 100 IU penicillin and 100 µg/ml streptomycin (23). Oocytes collected by one of the following techniques

1. Aspiration technique

The follicles with 2-15mm diameter aspirated by 10ml disposable syringe contain 2ml of PBS+10%FCS attached to 18(1) or 22(24, 25) gauge needle.

2. Slicing technique

Ovaries were put in sterile petri dish contain 10ml PBS+FCS10% as , held with forceps and cut into small pieces by sterile blade attached handle blade and wait 10 minutes to let oocytes settle down(26).

3. Dissection technique

Ovaries put in sterile petri dish and dissected the surface of ovaries with the follicles by sterile blade attached handle blade and washing with 15 ml PBS and wait 10 minutes to let oocytes

sink(27).

In vitro Maturation of she-Camel oocytes

Only grades A and B oocytes selected. The oocytes washed three time by PBS and transfer it to maturation medium at 38.5C°, 5% CO2 and 90 humidity for 24 hours. The incubated plate well (each well contain 2ml media) examined under light microscope(28). Morphological cumulus expended give indicator for maturation and appearance the first polar body give a good criteria oocytes maturation in vitro (IVM) (29). Calculated the numbers of matured oocytes as follow: total No. of oocytes cultured / total oocytes matured X100 (30).

Statistical Analysis

Student one-way Anova by SPSS version26 2019.

Results and Discussion

Maturation oocytes collected by aspiration technique

Table (1) show the effect of Vit. C on oocytes maturation rate collected by aspiration technique. (99/110) 90% of collected oocytes cultured. Maturation rate, expended cumulus cells was 66.66% (66/99) and the appearance first polar body (F.P.B.) was 68.1% (45/66). The present study showed maturation medium supplement with 50µm/ml of vit. C higher rate than other groups with significant (P<5%), and maturation media supplement with 25, and 100 µm/ml gave higher rate than control group with significant (P<5%).

The current study is somewhat similar to what (31)) found, where they were added 100µg/ml of vitamin C to the culture medium of goat oocytes gave 80% the rate of oocytes maturation, while the current study gave 84% the level of the Maturation at 50µg/ml and 100µg/ml of vit.C the rate decreased to 64%. Perhaps the culture environment, working conditions or animal species led to the difference in results. (32) Recorded maturation rate of cow oocytes after supplementing the media with 200µg/ml of vitamin C was 77.9% lower than our study with 50µg/ml, this difference in the result may be the high dose of vit. C have a negative role on IVM lead to damage oocytes or increase apoptosis (33). The present study disagreed with (29) were showing maturation rate 81.93% higher than our study 64% after supplementing IVM media with 100µg/ml of vit. C may be the role of combination HCG +PMSG add to medium increased maturation rate of oocytes or fetal dromedary calf serum(34).

Table (1) effect of Vit. C on oocytes maturation collected by aspiration technique

Group	No. of Oocytes	Maturation		Other
		Exp. Cum. (%)	P.B. (%)	
Control	25	12(48) b	7(62.5) a	13
C25	24	17(70.8) ab	11(76.4) ab	7
C50	25	21(84) a	17(81.8) a	4
C100	25	16(64) ab	10(70.5) ab	9
Total	99	66	45	33

The superscripts a, b is consider significant at ($P < 0.05$). Other refer to unexpended cumulus, damage or degenerated oocytes. Exp. Cu. refer to expended of cumulus cells.

Maturation oocytes collected by slicing technique

Table (2) show the effect of Vit. C on oocytes maturation rate collected by slicing technique. (96/134) of collected oocytes cultured. The maturation rate expended cumulus was 61.4% (59/96) and the appearance P.B. 62.7% (37/59). The present study recorded maturation medium supplement with 50µg/ml of vit. C higher rate than other groups with significant (P<5%), and maturation media supplement with 25, and 100 µm/ml gave higher rate than control group with significant (P<5%).

The current study agreed with (35), how point that adding 50µm/ml vitamin C gave the highest percentage of maturation compared to the control group. (36), showed mixing 100 µg/ml of vitamin C with 100 µg/ml of vitamin E, the level of cow oocytes maturation increased, with a higher rate of 83.23% cumulus cell expansion and 61.25% of the polar body appearance compared with the control group, roughly consistent with the present study as it showed cumulus cell expansion. The cumulus-expended ratio was 79.16%, but the polar body appearance rate was 73.68% higher than the previous study(36). The same previous study(36) corresponds to the presnt study with the same ratio of appearance of the polar body as 61.25 and 64.24 respectively when adding 25 mg of vitamin C.

Table (2) effect of Vit. C on oocytes maturation collected by slicing technique

Group	No. of Oocytes	Maturation		Other
		Exp. Cun (%)	P.B. (%)	
Control	24	9(37.5) b	6(66.66) b	15
C25	24	14(58.3) ab	9 (64.24) b	10
C50	24	19(79.16) a	14 (73.68 a	5
C100	24	13(54.16) ab	8 (61.53) b	11
Total	96	59	37	41

The superscripts a, b is consider significant at (P < 0.05). Other refer to unexpended cumulus, damage or degenerated oocytes. Exp. Cu. refer to expended of cumulus cells.

Maturation oocytes collected by dissection technique

Table (3) show the effect of Vit. C on oocytes maturation rate collected by slicing technique. (146/103)of collected oocytes cultured. The maturation rate (expended cumulus) was 57.2% (59/103) and the appearance P.B. 55.93% (33/59). The present study recorded maturation medium supplement with 50µg/ml of vit. C higher rate than other groups with significant (P<5%), and maturation media supplement with 25 µg/ml gave higher rate than 100 µg/ml and control group with significant (P<5%) but no difference significant (P<5%) between 25 and 100 µg/ml in appearance of F. P.B.

(37) , had shown when 200 µg added to the culture medium of bovine oocytes, this led to

expansion of cumulus cells 51.17% that is almost similar the current study, when adding 100µg/ml 52% while the rate of appearance of the first P.B. in the present study is 72.2% higher than the previous study. The same previous study(37) showed when adding 50 µg of vitamin C led to expanding cumulus cells by 52.79% and the appearance of the P.B. this is 30.43% different from the current study where it has expanded cumulus cells 73.07% and polar body 79.1% (37).

The difference between our study and the previous study (37) may be the difference in working methods where they were used vitrified oocytes may have resulted in different results, and evidence the control group used non-vitrified acts that result higher than other groups.

Table (3) effect of Vit. C on oocytes maturation collected by dissection technique

Group	No. of Oocytes	Maturation		Other
		Exp. Cum. (%)	P.B. (%)	
Control	26	12(46.15) b	5(24) c	14
C25	26	15(57.7.9) ab	9(70) b	11
C50	26	19(73.07) a	12(79.1) a	7
C100	25	13(52) b	7(72.2) b	12
Total	103	59	33	44

The superscripts a, b is consider significant at ($P < 0.05$). Other refer to unexpended cumulus, damage or degenerated oocytes. Exp. Cu. refer to expended of cumulus cells.

Conclusion

Supplement the media with 50 µg/ml of vitamin C gave higher maturation rate (cumulus expended and first polar body).

Aspiration technique the best method to oocytes maturation.

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