Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

Research article

Influence of PMSG on in vitro oocytes maturation of Iraqi she- Camel

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Abstract

The aim of the present study was know the role of PMSG in invitro maturation of she-camel oocytes. Ovaries where collected from Afak slaughterhouse and transport by cool box contain normal saline 0.9% (20-25C°) supplement with (penicillin G sodium 10^4 IU, streptomycin sulfate10mg and Amphotericin B 0.025 mg) to laboratory of Al-Diwaniyah veterinary Hospital during 1-2 hours.

Ovaries with visible follicles aspirated by 22-gauge needle attached to 10ml syringe and slicing the ovaries after aspiration. Only type A and B selected and matured in maturation medium (M199-A) supplied with 0, 2, 4, 10 IU of PMSG and incubated in CO2 incubator at 5% CO2, 38.5 Cº, and 90% humidity for 24h. The results was Maturation media supplement with 0IU of PMSG gave lower rate 24% of expended of cumulus cell and 0% appearance of the first polar body (F.P.B) than other groups. However, 10 IU of PMSG gave higher maturation rate 65.3% cumulus expended and 64.7% appearance of the first polar body (F.P.B).

Keywords: In vitro, PMSG, She- Camel.

Introduction

Almost assistant reproductive techniques development very slowly in Camels compared with other farm animals like cow, sheep, goat etc… so used assistant techniques to increase offspring numbers and overcome on fertility disorder because the camel under natural condition sever from low fertility. 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 (1, 2).

IVM oocytes consider the key to IVF, maturation process occur under influence of hormones so several studies showed that as (3) and (4) showed cumulus cells expended occur under effect of FSH. Supplement IVM media with LH increased metabolism of glutamine (5). By other side (6) showed LH has positive role in development bovine embryo when supplement the media with LH. Previous study showed supplement IVM media with eCG or FSH increase blastocyst and cleavage stage of buffalo oocytes (7).

Our study suggests supplement the media with different concentration of PMSG to know the effect on in vitro oocytes maturation of Iraqi she-Camel.

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Materials and Methods

Materials

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, and Pregnant Mare Serum Gonadotrophin (PMSG, or eCG) from Syntex Company Argentina.

Methods

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 (8). Oocytes collected by slicing after Aspiration technique.

Aspirated the follicles with 22 gauge needle attached to 10ml syringe contain 2ml PBS+FCS10% and then slicing ovaries in clean petri dish contain 10ml PBS+FCS%, held with forceps and cut into small pieces by sterile blade attached handle blade and wait 10 minutes to let oocytes settle down.

In vitro Maturation of she-Camel oocytes

Only type 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. Morphological cumulus expended give indicator for maturation and appearance the first polar body give a good criteria oocytes maturation in vitro (IVM) (9). Calculated the numbers of oocytes matured as follow total No. of oocytes cultured / total oocytes matured X100 (10).

Statistical Analysis

Student one-way Anova by SPSS version26 2019.

Results

Grade A and B of collected oocytes cultured. The maturation rate (expended cumulus) was 46.6% (47/101) and appearance F.P.B. 51% (24/47). Our study showed maturation media supplement with 0IU of PMSG gave lower rate 24% cumulus expended and 0% appearance F.P.B. than other groups with significant (P<5%), while group supplied with 10IU gave higher rate 65.3% cumulus expended and 64.7% appearance of F.P.B. and supplied the medium with 2 IU gave (44% expended of cumulus cell and 45.5% F.P.B,). While, 4 IU gave (52% and 53.8 expended of cumulus cell and F.P.B.) respectively. Table1

Table (1) effect of PMSG on oocytes maturation collected by slicing after aspiration technique

Group	No. of Oocytes	Maturation		Other
		Exp. Cum. (%)	P.B. (%)	
Control	25	6(24) c	0 c	19
PMSG 2IU	25	11(44) b	5(45.4) b	14
PMSG 4IU	25	13(52) ab	7(53.8) ab	12
PMSG 10IU	26	17(65.3) a	12(64.7) a	9
Total	101	47	24	54

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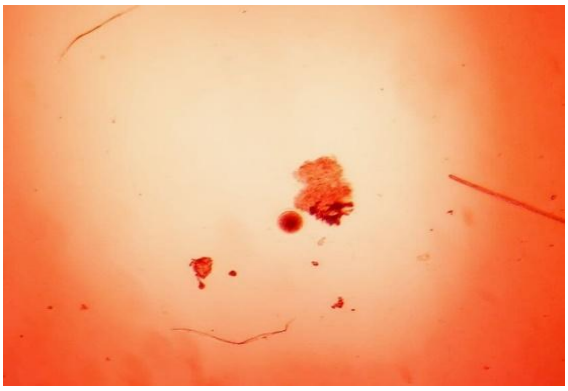
The superscripts a, b, and c are consider significant at (P < 0.05) at the same column. Other refers to unexpended cumulus, damage or degenerated oocytes. Different small letter mean significant difference (p< 0.05).



Complete expended cumulus cell

Appearance of the first polar body

Discussion

The present study showed similar almost with (11) when supplementing maturation media of sheep oocytes with 5, and 10µg of PMSG. Our result recorded the maturation rate was 44% and 52% after supplied the media with 2 and 4IU PMSG respectively, while the previous study recorded 45.8% with 5 µg, 51.7% with 20 µg and 46.4% with 10 µg of PMSG. Nevertheless, the present study recorded higher maturation rate 65.3% than previous study after supplied the medium with 10IU of PMSG compared with 20 µg, 51.7% and 30 µg, 45%. This is difference may be infertility, food, and the season of harvested oocytes.

(12) recorded when supplied TCM199 media with 10% stem serum and 20IU of PMSG gave 68.2% of cumulus expended of buffalo oocytes. this result almost similar to our study, when supplied the media with 10IU, while the same previous study showed when supplied the media with 2.5IU of PMSG, gave 22.2% of Cu. exp. this disagrees with our study when supplied with 2 or 4 IU of PMSG gave 44% and 52% respectively. The difference in results may be due to species or the supplementing material (12).

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