

Effect of alcoholic extract of Licorice (*Glycyrrhiza glabra*) on *in vitro* maturation of rabbits oocyte

دراسة تأثير اضافة مستخلص عرق السوس على انضاج بيوض الارانب مختبريا

Sadeq J. Zalzal Imad M.AL-Meeni Ihsan H. Al-Timimi

Department of Surgery and Obstetrics, College of Veterinary Medicine, Baghdad University

E.mail: sadeq.jaffar1@gmail.com

Abstract

The present study included 30 ovaries collected from 15 slaughter rabbits in animal house of the college of veterinary medicine, Baghdad University. Immediately, ovaries were preserved in phosphate buffer saline at 37 °C and transport to the laboratory with 1-2 hrs, oocytes collected by slicing ovary with an iris knife and release the follicular contents in 1ml TCM₁₉₉ media. Only oocytes surrounded by cumulus cells and homologs cytoplasm were culture in culture media, then oocytes were divided in 3 groups, the 1st group (control group) incubated by using culture media only, the 2nd group, alcoholic extract of Licorice added to culture media at concentration of 25µg/ml, the 3rd group culture media was supplemented with alcoholic extract of licorice at concentration of 50µg/ml. Results of the current study revealed that the effect of alcoholic extract 50 µg/mg on the percentage of oocytes maturation was superior compared with other groups. It was also observable that the effect of adding alcoholic extract of Licorice to culture media TCM₁₉₉ at two concentrations increased the percentage of mature oocyte compared with control group. In conclusion, the uses of alcoholic extract of licorice increase the percentage of oocytes maturation.

Key words: Licorice, Rabbit, IVM

المستخلص

شملت الدراسة 30 مبيضا لأرانب جمعت مباشرة بعد ذبح الحيوان بالبيت الحيواني في كلية الطب البيطري جامعة بغداد، حفظت المبايض في المحلول الفسلجي بدرجة حرارة 37 °م ونقلت الى المختبر مباشرة، تم حساب عدد الجريبات المتواجدة على كل مبيض تحت المجهر . جمعت البيوض بواسطة طريقه التقطيع لكل مبيض على حدة بواسطة المشروط الجراحي وتفرغ المحتوى الجريبي في اطباق زرعيه تحتوي 1 مل من الوسط الزرعي TCM₁₉₉ . البويضات المحاطة بالركمة المبيضية والسيتوبلازم المتناسق زرعت في الوسط الزرعي ثم قسمت البيوض الى ثلاثة مجاميع ، المجموعة الاولى تمثل مجموعة السيطرة استخدم فقط الوسط الزرعي TCM₁₉₉ لحضن البيوض، المجموعة الثانية اضيف مستخلص عرق السوس بتركيز 25 مايكروغرام/مللتر ، المجموعة الثالثة اضيف مستخلص عرق السوس بتركيز 50 مايكروغرام/مللتر. اظهرت نتائج الدراسة تفوق تأثير المستخلص الكحولي لنبات عرق السوس بتركيز 50 مايكروغرام/مللتر على النسبة المؤية لانضاج البويضات على المستخلص الكحولي بتركيز 25 مايكروغرام/مللتر، وسجل تأثير اضافة المستخلص الكحولي لنبات عرق السوس وبالتركيزين 50 و 25 مايكروغرام/مللتر زيادة لانضاج البويضات مقارنة بالوسط الزرعي (السيطرة). نستنتج من الدراسة الحالية ان استخدام المستخلص الكحولي لعرق السوس يزيد من النسبة المؤية لانضاج البويضات.

Introduction

Medical plants and herbs have been used for many years in treatment of various diseases in animals and human being. These are used in animals feed as the growth promoter (1). Licorice (*Glycyrrhiza glabra*) is one of the most popular plant and widely consumed as a medicinal herb, which is used mainly in treatment of inflammations and improvement of reproductive performance in both female and male animals (2). Licorice (*Glycyrrhiza glabra*) is one of the commercially important perennial plant grassy or semi-bushy type species from the leguminosae (Fabaceae) plant family, sub-family papilionaceae the genus name is driven from the Greek words , glycy (or glulus) means sweet and rhiza means root (3). Licorice roots, runners and rhizomes are the commercially desired parts of the plant that contain a number of important chemical compounds.

Glycyrrhizin is one of these compounds which shown to be 50 times or more sweet than sugar and demands high prices in world market (4).

Rabbit occupy a vital midway between ruminant and monogastric animals, they have been used as a model for human and other mammals in variety of researches in physiology biotechnology, toxicology, etc. They are medium size, readily accessible, easy to handle and easy to maintain (5). The application of assisted reproduction technology in rabbit such as induction of superovulation, *in vitro* maturation (IVM), *in vitro* fertilization (IVF) and Embryo Transfer (ET) will help increasing the number of offspring produced by genetically superior parents (6). The revised studies indicate that, through slicing, collecting greater number of oocytes than by follicular aspiration (7). There were no study used the Licorice extract have been used to improve *in vitro* maturation of rabbit oocytes, so this study was conducted to detect the effect of herbal extract (Licorice) on *in vitro* maturation of rabbit oocyte.

Materials and methods

Licorice herb root were collected from alshurja market, roots of the plants were identified by Dr. Ali Hussein AL-Musawi (Department of biology, College of Sciences University of Baghdad) as a Licorice plant root which belongs to leguminoceae (Fabaceae) plant family. Roots washed and left to dry in the air for 2 weeks at room temperature in the laboratory with continuous stirred. Dried roots were converted into fine powder by an electric grinder. The powder kept at 4°C until use. Fifty grams of licorice powder were extracted with 500 ml of 70% ethanaolic alcohol under continuous stirring for 8 hrs. at room temperature, the suspension was filtered by Whatman no. 1 filter paper, filtrate was concentrated by vacuum rotary evaporator (8). The crud extract was stored in dark sterile screw bottle at 4°C until use to prepare the required concentration.

Fifteen mature New Zealand white Rabbits were used in this study (weight 3-4 Kg. and aged 8-11 months). Does were caged under controlled temperature 20-27°C and humidity ranged between 70-80%, lighting (was 14hrs light and 10hrs darkness). Does fed commercial pellet diet (9). After 14 days of adaptation, does were intramuscularly injected with 20 IU/kg of eCG (Folligon®) (Intervet, Holland). Does were slaughtered 60-65hrs after hormonal treatment. Ovaries were transferred in a Petri dishes containing phosphate-buffered saline (PBS, Sigma, USA). Ovaries were washed 1 time with 70% ethanol then 3 times with saline solutions supplemented with 100 IU/ml penicillin and 100 µg/ml streptomycin (10).

From thirty ovaries counting number of preovulated follicles in individual ovary, oocytes collected in slicing method was performed in the ovary with an iris knife to release the follicular contents. After collection of oocytes from individual ovary were kept separated in 35 mm sterile disposable petri dishes using an orally controlled micropipette (11). Recovered oocytes examined under dissecting microscope and classified according to the morphology into 2 types, one characterized by being appraised fully compact cumulus cells and homogenous cytoplasm, while denuded oocytes with heterogeneous cytoplasm were rejected. Oocytes transferred to petri dish containing 100µl of culture media. This process repeated 3 times to wash the oocytes and get rid of the debris of the cumulus cell (12) . The oocytes collected by slicing were divided in to 3 groups:

- 1-First group (control group): Oocytes maturation applied in the culture media TCM₁₉₉ (Invitrogen, USA) supplemented with 10 IU/ml of eCG, 10 IU/ml LH (Chorullon®) (Intervet, Holland), 100 IU/ml penicillin, 20µg/ml streptomycin sulphat and 10% fetal calf serum (stock media), and without adding extracts of licorice.
- 2- Second group: Maturation of oocytes applied in the culture media TCM₁₉₉(stock media), with the addition of alcoholic extract of the licorice 25µg/ml.
- 3- Third group: Maturation of oocytes applied in the culture media TCM₁₉₉(stock media), with the addition of alcoholic extract of the licorice 50µg/ml.

Where been confinement of pH degree by the PH meter and the osmotic pressure of culture media of the three groups of experiment, and covered surface with adequate quantity of mineral oil. Then incubated in petri dish in CO₂ incubator at 38°C, 5% CO₂ and 100% humidity. After 24 hrs. of incubation oocytes were examined for maturation by inverted microscope where the oocytes

stripped from cumulus oophorous by aspiration gently and rewind several times using a micro-pipette, after that classified to mature and immature according to the existence of the first polar body in the prevetlin space of mature oocyte(13).

Statistical Analysis

The Statistical Analysis System- SAS (2012) was used to effect of different factors in study parameters (14).

Results and Discussion

Thirty ovaries which were collected from 15 does slaughter after super ovulation respond to increase follicles on the ovaries then increase oocytes collected after slicing ovaries. table (1) illustrate the 518 preovulatory follicles from 30 ovaries and mean number of 17.26 follicles, this result agree with (15) when rabbits administration of 150 IU PMSG induce satisfactory follicles response in both breeds , and thus breed influence could not be appreciated in superovulation response. From 518 preovulated follicles 471 oocytes collected by slicing method, the mean number of collected ova rate 90.29 % this result corresponded with Lorenzone, et.all.(1996)(11) , show no difference in number of recovered oocyte and recovery rate (oocyte obtained /follicle selected x100) were found between the slicing and aspiration method .

Table (1). The relationship between the follicle in the superovulated rabbit ovary and oocyte collected in slicing method.

Number of ovaries	Number of follicles	Number of oocytes
10	180	162
10	166	152
10	172	157
30	518	471
	17.26%	90.92%

There was significant increase in the mature oocytes ($p \leq 0.1$) by using 50µg/ml of Licorice extract in comparative with control group (table2). While in concentration 25µg/ml there was no significant different in the percentage of mature oocytes compared with the control group. Also table(2) demonstrates that the percentage of immature oocytes were significantly ($p \leq 0.01$) lower on the alcoholic extract 50µg/ml compared with control group. The present study could be considered as advance research focusing on benefit of this herb in laboratory maturation of oocyte. This results correspond with those of (16) who concluded that the addition of Glycyrrhiza glabra extract in the culture media of sperm and oocytes can enhance the fertilization, embryonic development and embryonic quality in mice. *In vivo*, the consummation of licorice increase ovulation rate and successful implantation of fertilized ova (17), and this means consequently the good level of FSH and LH may be available since these hormones are responsible for stimulation follicle development and ovulation (18).

Table (2): Effect of various concentration of alcoholic extract of Licorice on rabbit oocytes maturation.

Parameter	Number of oocytes	Mature oocytes		Immature oocytes	
		number	%	number	%
Control media	162	120 B	74.07%	42 A	25.93%
Alcoholic extract 25µg/ml	152	118 B	77.63%	34 A	22.37%
Alcoholic extract 50µg/ml	157	131 A	83.43%	26 B	14.47%

*Different Capital letter groups means significant difference ($p \leq 0.01$) Between groups

References

1. Tipu, M.A.; Akhtar, M.S.; Anjum, M.I. and Raja, M.L. (2006). New dimension of medicinal plants as animal feed. Pakistan Vet. J. 26 (3): 144-148.
2. AL-Jiboori, B.M. and Faliah, N.K. (2010). Developmental Change in the Genital Organs of Young Mal Mice Associated with Licorice Extract Consumption by Mother before and during Gestation .Iraq j. Med. Sci. 8 (3):14-19.
3. Crusheva, R. and Parvanov, R. (1978). Album of protected plants. Bulgaria: pp. 27-28
4. Olukoga, A. and Donaldson, D. (1998). Historical perspective on health. The history of Licorice: the plant, its extracts, cultivation, commercialization and etymology. J. Roy. Soc. Health .118 (5): 300-304
5. Bosze, Z.S. and Houdebine, L. M. (2006). Application of rabbits in biomedical research: review. World Rabbit Sci. 14:1-4
6. El-Maghraby, E.F.A., (2004). Some factors affecting *in vitro* maturation of ovine oocytes M.SC Thesis Animal production Department, Ain Shams University.
7. Ariotto, T.M. ; Lebfried-rutiedge, M.L. and FirstN.I. (1990). Size distribution and meiotic competence of bovine primary oocyte from tow localization in the ovary. Theriogenology .33. 188
8. Montmat, L.A.; Fag, C.A. and Petra, O.M. (1982). Effect of cotton extract on sperm morphology. Andrologia, 101-105.
9. Quientela, I.A.; Pera A.I.; Vega M.D.; Gullon, J., Perioto C.; Barrio, M.; Becerra J.J.; Maseda, F. and Herradon, P.G. (2004). Ovulation induction in rabbit does submitted to artificial insemination by adding buserelin to the seminal dose. Reprod. Nutr. Dev., 44:79-88.
10. Ahmed, N.A.; Fawzy, S.A.H.; Ganah, H.A.B.; Imam, A.M., and Mehaisen, G.M.K. (2008). In vitro maturation of rabbit oocytes supplemented with leptin. EGY. CON. on RAB. SCI. 1: 124-130
11. Lorenzo, P.L.; Rebollar, P.G.; Lllera, M.J.; Lliera, M. and Alvarino, J.M.R. (1996). Characterization of Rabbit follicular oocytes and their ability to mature in vitro. Arch. Zootec. 45: 25-35
12. AL-Maeni, I.M. ; AL-Samaree,K.W. and Abdul rhman,L.Y.(2012). Study the effect of addition alcoholic extract of Tribulus terrestris on bovine oocyte maturation in vitro.Iraqi j.Vet.Med.36(2):199-203
13. Abdel-Khalek, A.E.; Dora, T.M. ; Shamiah, S.M. and AL-RateI.T.(2012). In vitro fertilization and development of in vitro versus in vivo matured rabbit oocytes. Egyptain j.Anim. Prod.49(3):321-327
14. SAS. (2012). Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.
15. Satheshkumar, S. (2006). Superovulation and embryo recovery in two breeds of rabbits. Ind. Jor. of Bio. 5 (1): 54-57
16. Sultan, A. S. H. (2008). The effect Glycyrrhiza on the *in vitro* fertilization in mice. MSC Thesis, biotechnology research center, AL-Nahrain University, Iraq
17. Ridaha, F.E.(2008) Effectof licorice extract (Glycyrrhiza glabra) on some fertility aspects of local Iraqi Does. MSC Thesis , Department of Surgery and Obstetrics, College of Veterinary Medicine, Baghdad University.
18. Costanzo, L. S. (1998). Reproductive physiology. In text book of physiology, W.B. Saunders company, Philadelphia. 404.