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EFFECT OF LICORICE EXTRACT (GLYCYRRHIZA GLABRA) ON *IN VITRO* FERTILIZATION OF IRAQI LOCAL GOAT OOCYTES

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Abstract

This study was conducted on 82 ovaries were collected from 41 slaughter local Iraqi goats in the Al-Shula abattoirs/Baghdad province, their ages vary from 3 to 5 years. Oocytes collected by slicing ovary, Only grade A and grade B (oocytes surrounded by cumulus cells and homologs cytoplasm) where cultured in culture media, then oocytes were divided in 3 groups, the 1st group (control group) incubated by using Tissue culture medium 199 (TCM-199) only, the 2nd group 'alcoholic extract of Licorice added to culture media (TCM-199) at a concentration of 25µg/ml, the 3rd group culture media (TCM-199) was supplemented with an alcoholic extract of licorice at a concentration of 50µg/ml. Results of the current study revealed that the significant different in the mature oocytes ($p \leq 0.1$) by using 50µg/ml of Licorice extract in comparative with concentration 25µg/ml and control group, Also there was significant different in the fertilization oocytes ($p \leq 0.1$) by using 50µg/ml of Licorice extract in comparative with concentration 25µg/ml and the control group. They attributed these results to the constituents of Licorice extract such as vitamins, minerals, estrogenic, anti-estrogenic substance, trace element, polysaccharide and antioxidant compounds which affect successfully maturation and fertilized of oocytes.

key words : Licorice, goats, IVF, TCM.

Introduction

Goats are vital livestock needed for a multiplied purpose, such as milk and meat (Dhanda *et al.,* 2003). Several studies have expounded that live young caprine can be created from in vitro and in vivo production of caprine embryos (Malaker *et al.,* 2007). The production of numbers of embryos by IVP in an animal can improve the understanding of biological processes such as the embryo implantation and the molecular switch and endocrine controls of oocytes maturation (Galli *et al.,* 2003). In most researches, they supplemented the media with herbs and hormones. It is necessary that the supplement of the in vitro maturation be like the in vivo environment. The selections of supplements for IVM are essential for subsequent IVF and in vitro developments (Pawshe *et al.,* 1996). There are many medicinal herbs can develop the reproductive performance of the female and male genital system. (AL-Jiboori, *et al.,* 2010 and Salah, 2015). One of these medicinal herbs is Licorice (*Glycyrrhiza glabra*) that have many important nutritional components (Grieve, M.1995). Its act as anti-inflammatory, antioxidant, antiviral, anti-allergic (Taylor, L. 2004). Licorice extract improved sexual activity and semen quality in Awassi rams and decreased the age of puberty in Awassi lamb rams (Al-Shammary, *et al.,* 2013) In Local Iraqi does used to Induction of true Estrous and Super ovulation (Redaa, *et al.,* 2010). There were no study used the alcoholic extract of Licorice to improve in vitro fertilization of goats oocytes, so the aim of this study was to investigate the effect of adding alcoholic extract of Licorice to culture media TCM at two concentrations on the oocytes maturation and fertilization in caprine.

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Materials and Methods

They conducted this study in the College of Veterinary Medicine (University of Baghdad)

Sperm preparation

Testis obtained from Al-shu'alah abattoir and transported by cool box to the laboratory within 2-4 hours. Sperms were harvested by slicing cauda epididymis using normal saline (Saleh, 2016). Evaluation of samples was under a microscope, samples were rejected if the

individual motility lowers than 60%.Incubation in 5% CO2 at 35 R"C for 6hrs for sperm maturation, distal protoplasmic droplet it presence if the sperm maturation (Palamo *et al.,* 1999).

Capacitation of sperms

Incubated for 45 minutes at 38 R"C according to the procedure described by Palamo *et al.,* (1999).

Oocytes collection

They collected caprine ovaries from Al-shu'alah abattoir and carried away to the laboratory within 2-4 hours by cool box. A sterile scissor removed the tissue surrounding ovaries. They washed each ovary in normal saline. They placed ovaries in a Petri dish having a normal saline and chopped into small pieces by a surgical blade. The cumulus-oocytes complexes (COCs) were selected from the saline solution. (Wang *et al.,* 2007).

Grading of oocytes

They examined collected oocytes under a microscope and graded according to Wani et al. (2000) as good (grade A), fair (grade B) and poor (grade C), based on cumulus cells and uniform cytoplasm.

Good: Oocytes with many complete layers of cumulus cells and uniform cytoplasm.

Fair: Oocytes with incomplete layers of cumulus cells and uniform cytoplasm.

Poor: Oocytes with few or no cumulus cells with fragmented cytoplasm.

***In vitro* maturation**

Only good(grade A) and fair (grade B) classified oocytes were selected, washed twice in maturation medium (TCM), and incubated at 39 C, 5% CO2 and 90% relative humidity for 24-28 hrs. The presence of the first polar body was a good decisive factor for the maturation of oocytes *in vitro* (IVM), the numbers of mature oocytes were calculated (Kharche *et al.,* 2011).

***In vitro* fertilization**

They diluted capacitated sperms to a concentration of 1.0×106 sperm/ml in the fertilization medium (TCM). Only matured oocytes were kept (10 in Petri dish) containing fertilization medium with sperms and incubated at 39 C, 5% CO2 and 90% relative humidity for 24-27 hrs (Kharche *et al.,* 2011).

Evaluation of fertilized oocytes

They evaluated oocytes having a second polar body as fertilized oocytes. They counted the numbers of fertilized oocytes (Kharche *et al.,* 2011).

Results and Discussion

(Table 1) shows the total number of goats oocytes used was 435 divided as matured oocytes 263 (60.41%) and Immature oocytes 172 (39.58%) there was significant different in the total mature oocytes (pd"0.1) comparative with total Immature oocytes and also there was a significant other in the mature oocytes ($p \leq 0.1$) by using 50µg/ml of Licorice extract in comparative with concentration 25µg/ml and the control group. While in concentration 25µg/ml there was significant different in the mature oocytes compared with the control group. (Table 2) shows the total number of fertilized oocytes was 168, the fertilization rate of Alcoholic extract 25mg/ml oocytes was (64.83%) 59/91 and the fertilization rate of Alcoholic extract 50mg/ml oocytes was (72.44%) 71/98 but in Control group was (51.35%) 38/74 there was significant different in the fertilization oocytes ($p \leq 0.1$) by using 50µg/ml of Licorice extract in comparative with concentration 25µg/ml and the control group. While in concentration 25µg/ml there was significant different in the fertilization oocytes compared

Table 1: Effect of various concentrations of alcoholic extract of Licorice on oocytes maturation.

Parameters(groups)	No. of oocytes	Mature oocytes		Immature oocytes	
		No.	%	No.	%
Control	142	74	52.11aC	68	47.89bA
Alcoholic extract 25mg/ml	149	91	61.07aB	58	38.92bB
Alcoholic extract 50mg/ml	144	98	68.05aA	46	31.94bC
Total	435	263	60.41a	172	39.58b

Different small letters mean significant differences (P<0.01) within groups.

Different capital letters mean significant differences (P<0.01) between groups.

Table 2: Effect of various concentration of alcoholic extract of Licorice on oocytes fertilization.

Parameters(groups)	No. of oocytes	Mature oocytes		Immature oocytes	
		No.	%	No.	%
Control	142	74	52.11aC	38	51.35c
Alcoholic extract 25mg/ml	149	91	61.07aB	59	64.83b
Alcoholic extract 50mg/ml	144	98	68.05aA	71	72.44a
Total	435	263	60.41	168	62.87

Different small letters mean significant differences (P<0.01) within groups.

Different capital letters mean significant differences (P<0.01) between groups.

with the control group. These results in table 1 and 2 show adding of alcoholic extract of Licorice to the culture media enhances maturation and fertilization rate of goats oocytes and this may be because of Licorice extract which has a wide range of minerals and vitamins such as vitamin E, vitamin C, zinc, selenium, glutathione, Hypodorian, taurine, beta carotene and carotene which may play a critical role in conversion of ROS to H2O (Van Langendonck *et al.*, 2002 and Agarwal *et al.*, 2003). Vitamin E and vitamin C is a chain-breaking antioxidant that stops the propagation of the per oxidative process, also Licorice extract has potassium, Ca+2, Zn+2, fructose, glucose, sucrose, amino acid, and many other substances (Taylor, L. (2004), all these substances increased sperm motility and the grade activity of forward movement(HARAGUCHI, H *et al.*, 2000). Oxidative stress may affect the oocyte and embryo. so, culture media the exogenous site of oxidative stress generation, affecting the oocytes and embryo (Harvey, A. J., Kind, K. L., & Thompson, J. G. (2002). Licorice extract has antioxidant compounds like: hispaglabridin A and B, isoflavone, which inhibit Fe + 3 induced mitochondrial lipid peroxidation, may be improved the total antioxidant capacity in TCM that later successfully maturation and fertilized of oocytes. (McKiernan, S. H., & Bavister, B. D. 1990).

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