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Original Article Research

EFFECT OF SOME PLANT OILS ON REPRODUCTIVE ACTIVITIES IN FEMALE ALBINO RATS

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ABSTRACT

The present study aimed to determine the effect of adding plant oils; extra virgin olive oil (EVOO), sunflower and soybean to animal feed on serum estradiol (E_2) and progesterone (P_4) levels, histological structure of ovaries and in vitro maturation of oocytes (IVM). A total of 60 mature female Albino rats were used. Animals were divided equally into 5 groups; control group (received standard diet), group II (received EVOO), group III (received sunflower oil), group IV (received soybean oil) and group VI (received oil mixture which consist of sunflower and soybean oils). After 6 weeks of feeding oil addited diet, blood samples were collected from all rats throughout the different stages of estrous cycle. Sera were used for determination of serum E_2 and P_4 levels. Only females that were not in estrus were scarified after the last blood sample collection, ovaries were harvested for histopathological examination and for in vitro maturation.

Results showed that none of oils led to ovarian changes except soybean oil and oil mixture, cause congestion of some ovarian blood vessels. It was also noted that the hormonal pattern didn't differ significantly among different treatments within the same stage of the cycle, except for the group received oil mixture where E_2 and P_4 levels decreased significantly ($P < 0.05$) during metestrus and diestrus phases, respectively. In the treated groups, the highest significant ($P < 0.05$) oocyte recovery rate (RR) (5.43 ± 0.23% and 4.41± 0.13%) and maturation rate (MR) (79.17 ± 2.03% and 73.43 ± 1.97%) were attained after application of EVOO followed by sunflower oil, respectively. While the lowest values were calculated with the soybean oil and oil mixture (3.83 ± 0.13 % and 2.50 ± 0.16 %) and (68.18 ± 2.29 % and 62.50 ± 2.23 %), respectively. It could be concluded that EVOO as well as sunflower oil have a beneficial influence on ovarian functional performance, retrieval of high number of good quality oocytes and raise oocyte maturation.

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INTRODUCTION

During the last few decades, scientists have paid their attention to health effects concomitant with adding some types of natural oils such as olive oil, sunflower oil and soybean oil to animal feed **(Mckevith, 2005)**. The value of oil is related mainly to its fatty acid composition **(Dyer et al., 2008)**. Extra virgin olive oil (EVOO) was found to control lipoprotein profile, blood pressure, antithrombotic activity and glucose metabolism **(Perez-Jimenez et al., 2005)** as well as it protects against colon and breast cancers **(Psaltopoulou et al., 2004)**. In addition, it protects blood lipids against oxidative damage and reduces coronary heart diseases due to its high oleic acid content and other bioactive components; such as polyphenols, vitamin E and hydrocarbons, that have anti-oxidative, anti-ischemic **(Visioli et al., 2002)** and anti-inflammatory properties **(Martin-Pelaez et al., 2013)**. Concerning sunflower oil, reports showed that it possesses skin-health benefits **(Pal, 2011)**, anti-inflammatory effect and improves lipid profile **(Masi et al., 2012)** as well as protects against cardiovascular diseases **(Binkoski et al., 2005)**. In this respect, it contains high levels of vitamin E **(Idson b, 1993)**, which has a prominent antioxidant activity and hypocholesterolemic effect, beside other oil components such as linolenic fatty acids and linoleic (LA) **(Booyens et al., 1988)**. Moreover, soybean oil is an important source of vitamin E that helps to lower serum cholesterol and low density lipoprotein "LDL" levels and prevents atherosclerosis **(Gresshoff, 2013).** Additionally, it was showed that soybean oil has the power to minimize oxidative stress by elevation of natural enzymatic antioxidants as superoxide dismutase, glutathione peroxidase and catalase **(Papazzo et al., 2011)**. Also, it regulates adipokines and proteins involved in adipose tissue metabolism and inflammation **(Chuffa et al., 2015).** Moreover, it was recorded that low amount of soybean oil, rich in both linolenic and LA, ameliorates the diabetic phenotype, protects pancreas from oxidative damage and restores Δ6 desaturase levels which is the key enzyme in the metabolism of essential fatty acids. Soy oil contains isoflavones which help in preventing osteoporosis and menopausal symptoms as well as reducing the risk of uterine cancers by blocking the estrogen receptors activation **(Song et al., 2007)**. There is a lack of evidence about the influence of these oils on reproduction in this context. **Reed et al. (1987)** showed that olive oil increases E_2 and P_4 levels before mating and at the end of lactation period and increases prolactin hormone levels that plays a role in fertility and allows fertilized eggs to develop and mature. Moreover, **Salem (2015)** recorded that EVOO improves the health status during pregnancy and lactation periods, reduces the risk of female infertility and increases the number and quality of foeti. **Vishnu et al. (2017)** stated that EVOO affects follicular development through hormones acting on the ovarian level such as E_2 and P_4. Regarding sunflower oil **Balevi et al. (2003)** and **Midilli et al. (2009)** recorded that administration of this oil in the diet improves reproductive performance through improving fatty acid composition of yolk, which in turn improve embryonic development, fertility, hatchability and increase feed conversion rate of quail. **Safdar et al. (2017)** mentioned that sunflower oil has a role in synthesis and secretion of prostaglandin E2 (PGE2) which is considered a critical mediator of oocyte maturation and it is important for maintenance of pregnancy in ewes during flushing period. Concerning soybean oil, it was recorded that consumption of soybean products in female rodents causes alterations in ovarian development, timing of vaginal opening, estrous cyclicity, ovarian function, HPG axis and increased incidence of uterine adenocarcinoma as well as subfertility **(Chen et al., 2007; Delclos et al., 2009 and Serag El Din et al., 2011)**. These bad effects could be attributed to their content of isoflavones and it contain high levels of linolenic acid (omega-3) that alters oocyte recovery rate, decreases the number of obtained oocytes and decreases the maturation rate through decreasing the number of oocytes reaching MII with a reduction in cumulus expansion **(Wakefield et al., 2008; Marei et al., 2010 and Serag El Din et al., 2011)**. Moreover, mice fed a high omega-3 diet for 4 weeks caused alteration in fatty acid content in the ovary and this was associated with altered oocyte mitochondrial distribution, increased reactive oxygen species (ROS) levels, poorer embryo morphology and development into blastocyst following fertilization **(Wakefield et al. 2008)**. Data concerning the influence of the aforementioned oils on in vitro embryo production (IVEP) seems to be scanty. Therefore, the current study aimed to determine the influence of adding different oils to diet on gonadal steroid hormones, quantity and quality of recovered oocytes and their in vitro maturation in female rats.

MATERIAL and METHODS

A) Animals :

The present study was implemented in the Animal Experimental Unit Department of Physiology, Faculty of Veterinary Medicine, Beni-Suef University, during December 2017 till the end of February 2018. Sixty mature clinically healthy, cycling female Albino rats (150 – 180 g BWT) were used. Rats were left for 2 weeks for acclimatization. Rats were housed in cages in a room at 25 °C with controlled humidity on a 12 h light: 12 h dark cycle and kept under hygienic conditions and offered balanced diet and water ad libitum.

B- Chemical analysis of plant oils used in the current study:

Acidity % and peroxide value for all oils (Table.1) were measured according to **AOAC (2005)**. Fatty acid composition of oil mixture (Table.1) was measured according to **ISO 12966-2 (2011)**. All measurements were done in Food Technology Research Institute, Agriculture Research Center, Egypt. Data for fatty acid composition of EVOO was recorded as stated by **IOOC (2003)**, while that of sunflower and soybean was recorded as stated by **Orthoefer (1996)** (Table.1).

C)Preparation of the oil addited diet :

Each type of oil was mixed thoroughly at a rate of 10 % of the ration. To minimize oxidation, all diets were prepared once weekly and stored at 4ºC in the refrigerator **(Ruiz-Gutie´rrez et al., 1999)**.

D) Experimental design :

Rats were equally divided into 5 groups (12 rats/group); control group (received standard diet), group I (received EVOO), group II (received sunflower oil), group III (received soybean oil) and group IV (received mixture of sunflower and soybean oil). After 6 weeks of feeding oil addited diet, blood samples were collected from all rats throughout the different stages of estrous cycle, At least, five estrous cycles from each rat were included.

Blood sample was collected from the orbital venous plexus of the rat between the hours of 06:30 and 09:30 before access to food and water under mild ether anesthesia **(Biegel et al., 1998)**. Sera were separated and kept at - 20˚C till hormonal assay.

Only the females that were not in estrus were scarified after the last blood sample collection, ovaries were harvested; 20 ovaries were used for in vitro maturation and the remaining for histopathological examination.

All procedures were performed in strict accordance with the recommendations and ethical guidelines for the care of animals used for experimental and other scientific purposes according to the Institutional Animal Care and Use Committee of Beni-Suef University, Beni-Suef, Egypt.

The obtained data were subjected to statistical analysis using ANOVA as described by **Snedecor** and **Cochran (1987)** and **SAS Program (1994)**.

Table 1: Chemical analysis of plant oils:

Item		EVOO	Sunflower	Soybean	Mixture
Acidity% (as oleic acid)		0.76	0.07	0.131	0.135
Peroxide value (Meq.O2/Kg oil)		9.85	5.99	1.39	5.39
Fatty acid composition	**Myristic acid(C14:0)**	≤ 0.05	0.2	0.1	0.08
	Palmitic acid (C16:0)	7.5-20	6.8	11.0	9.90
	Palmitoleic acid(C16:1)	0.3-3.5	0.1	0.1	0.10
	Stearic acid (C18:0)	0.5-5	4.7	4.0	4.04
	Oleic acid (C18:1)	55-83	18.6	23.4	24.45
	Linoleic acid(C18:2)	3.5-21	68.2	53.2	54.46
	Linolenic acid(C18:3)	< 1.0	0.5	7.8	5.71
	Arachidic acid (C20:0)	< 0.6	0.4	0.3	0.33

RESULTS

1) Effect of different oil treatments on the estrous cycle :

In the current study, vaginal smears revealed that none of the applied oil led to changes in the cellular characteristics of the expected phases of the estrous cycle as compared with those of the control group.

2) Effect of different oil treatments on Estradiol level (E_2) and Progesterone level (P_4) during different phases of estrous cycle:

Results of the present study clarified that, throughout the experimental period, in control as well as oil administered females, the highest E_2 levels were recorded at proestrous and estrous phases (68.75 ± 6.14 pg/ml and 57.19 ± 5.17 pg/ml, respectively) while, for the highest levels for P_4 were recorded at postovulatory stages (metestrus and diestrus) of the estrous cycle (17.63 ± 1.32 ng/ml and 12.98 ± 0.63 ng/ml, respectively) (Table.2 and 3). It was also noted that the hormonal pattern didn't differ significantly among different treatments within the same stage of the cycle, except for the group received oil mixture where E_2 and P_4 levels decreased significantly ($P \leq 0.05$) during metestrus and diestrus phases, respectively.

3) Effect of different oil treatments on recovery rate and maturation rate of oocytes:

the highest significant ($P < 0.05$) oocyte recovery rate (RR) (5.43 ± 0.23%, 4.41± 0.13%) and maturation rate (MR) (79.17 ± 2.03%, 73.43 ± 1.97%) were attained after application of EVOO followed by sunflower oil, respectively. While the lowest values were calculated with the soybean oil and oil mixture (3.83 ± 0.13 %, 2.50 ± 0.16 %) and (68.18 ± 2.29 %, 62.50 ± 2.23 %), respectively. It was also noted that there were no significant difference in RR and MR between control and sunflower oil treated groups (Table.4).

4) Histopathological findings:

Histopathological findings of the ovarian sections of the control as well as EVOO and sunflower oil treated groups disclosed normal ovarian structure with different stages of mature Graafian follicles and multilayered follicular epithelium and corpus luteum (Figs. 1, 2 and 3). On the contrary, administration of soybean oil alone resulted in severe congestion of the ovarian blood vessels in the medulla with different stages of mature Graafian follicles and corpus luteum (Figs. 4) and when mixed with sunflower oil led to severe congestion of ovarian blood vessels with few number of mature Graafian follicles and many corpora lutea (Figs 5).

Table 2: Estradiol level (pg/ml) during different phases of estrous cycle (Mean ± SE).

Items	Control	EVOO	Sunflower	Soybean	Mixture
Proestrus	68.75 ± 6.14^{A}	67.95 ± 4.78^{A}	66.09 ± 5.19^{A}	72.54 ± 5.13^{A}	65.33 ± 4.22^{A}
Estrus	57.19 ± 5.17^{A}	53.16 ± 3.44^{A}	55.22 ± 5.45^{A}	60.42 ± 5.14^{A}	64.52 ± 5.13^{A}
Metestrus	32.53 ± 3.39^{B}	30.15 ± 2.98^{B}	31.64 ± 4.14^{B}	35.31 ± 3.81^{B}	23.19 ± 1.56^{B}*
Diestrus	21.11 ± 4.05^{C}	22.17 ± 2.14^{C}	20.21 ± 2.42^{C}	28.14 ± 3.35^{C}	$24.64 \quad 2.23^{B}$

-SE: Standard error.
-In the same estrous cycle, values having different letters differ significantly from each other at ($P \leq 0.05$).
-In the same stage, values with stars (*), differ significantly ($P \leq 0.05$) from the corresponding.

Table 3: Progesterone level (ng/ml) during different phases of estrous cycle (Mean ± SE).

Items	Control	EVOO	Sunflower	Soybean	Mixture
Proestrus	3.45 ± 0.30 ^{A}	4.01± 0.51 ^{A}	4.17±0.18 ^{A}	3.36±0.41 ^{A}	3.96±0.45 ^{A}
Estrus	4.01 ± 0.37 ^{A}	5.22 ± 0.55 ^{A}	4.81±0.43 ^{A}	4.56±0.36 ^{A}	4.03±0.47 ^{A}
Metestrus	17.63 ± 1.32 ^{B}	18.02 ±1.59 ^{B}	16.91±0.98 ^{B}	17.53±0.98 ^{B}	16.07±1.77 ^{B}
Diestrus	12.98 ± 0.63 ^{C}	12.09 ±1.03 ^{C}	11.91±1.57 ^{C}	10.77±1.31 ^{C}	10.08±0.95 ^{C*}

-SE: standard error
-In the same estrous cycle, values having different letters differ significantly from each other at ($P \leq 0.05$).
-In the same stage, values with stars (*), differ significantly at ($P \leq 0.05$) from the corresponding.

Table 4: Effect of oils administration on recovery rate and maturation rate of oocytes (Mean ± SE).

Items	Control	EVOO	Sunflower	Soybean	Mixture
Total number of ovaries	20	20	20	20	20
Number of oocytes	96	108	88	76	56
Recovery rate	4.83 ± 0.18 ^{a}	5.43 ± 0.23 ^{b}	4.41 ± 0.13 ^{a}	3.83 ± 0.13 ^{c}	2.50 ± 0.16 ^{d}
Good quality oocytes	81	96	64	66	40
Total number of mature oocytes	60	76	47	45	25
Maturation %	74.07 ± 1.18 ^{a}	79.17 ± 2.03 ^{b}	73.43 ±1.97 ^{a}	68.18± 2.29 ^{ac}	62.50 ± 2.23 ^{cd}

-SE: Standard error.

-In the same row, values having different small are significantly at $P \leq 0.05$.

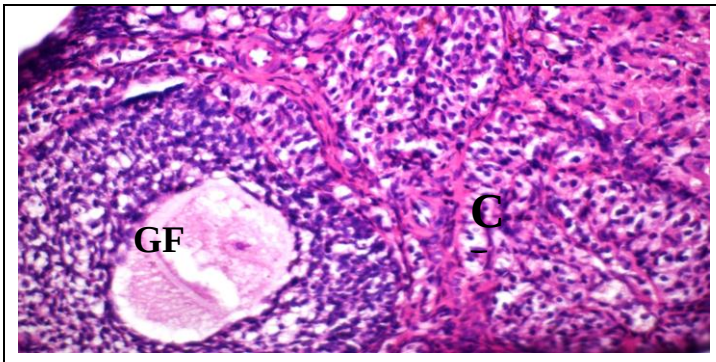

Fig. 1: Ovary from control group showing normal ovarian structure (H and E, microscopic magnification "mm" 400).

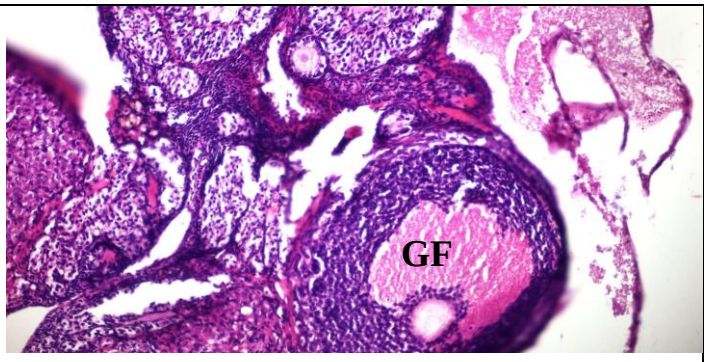

Fig.2: Ovary from olive oil group revealed normal structure with different stages of ovarian follicles with multilayered follicular epithelium. Some follicles contain ova with covering epithelium projecting into the antrum. Corpora lutea are seen in the ovarian cortex (H and E, microscopic magnification "mm" 200).

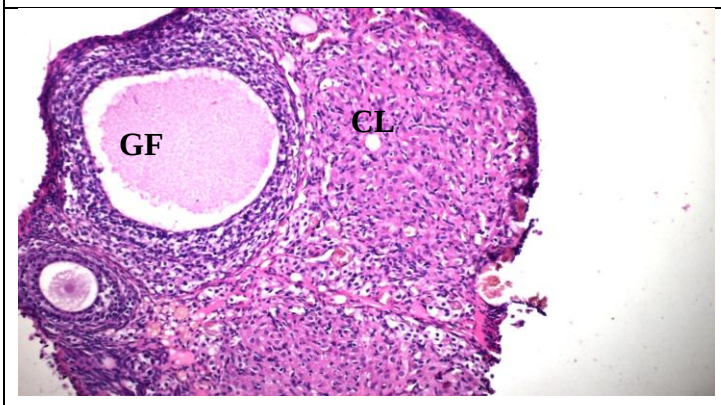

Fig.3: Ovary from sunflower oil group revealing normal ovarian structure with different stages of mature Graafian follicles and multilayered follicular epithelium and corpus luteum (H and E, mm 200).

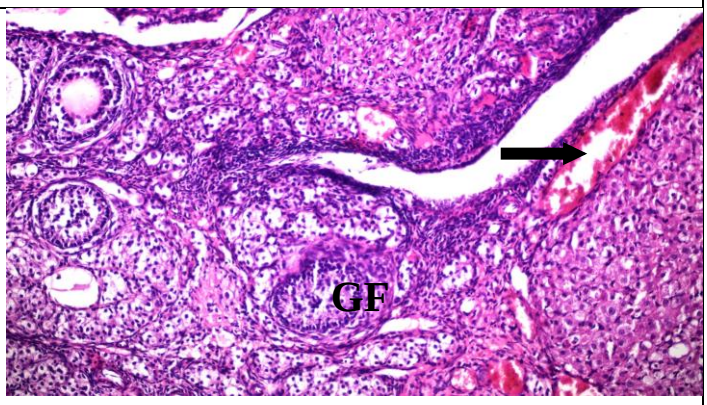

Fig.4: Ovary from soybean oil group demonstrating congestion of the ovarian blood vessels in the medulla with different stages of mature Graafian follicles and corpus luteum. (H and E, mm 200).

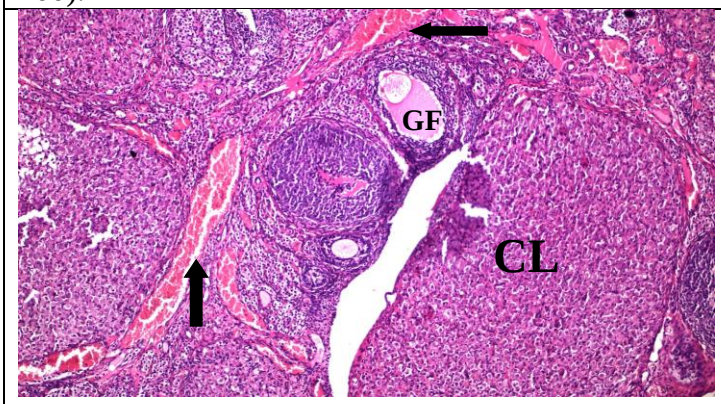

Fig. 5: Ovary from group received oil mixture showing severe congestion of ovarian blood vessels with few number of mature Graafian follicles and many corpora lutea (H and E, mm 100).