



EFFECTS OF EWE COLOSTRUM EXTRACT (COLLECTED AT ZERO-HOUR AND 12-HOUR AFTER PARTURITION) ON THE BLOOD BIOCHEMICAL PARAMETERS AND THE SERUM LEVELS OF FSH, LH AND TESTOSTERONE IN MALE RATS (*RATTUS NORVEGICUS*)

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Abstract

The present experimental was focused on exploring the effects of the ewe colostrum extract (ECE) on the overall performance and the serum levels of FSH, LH and testosterone in male rats (*Rattus norvegicus*). The ECE was produced from colostrum collected at zero-hour and 12-hour after parturition. The experiment was performed using 30 male rats (*Rattus norvegicus*) that were randomly separated into three groups (10 animals/group); a group that received 0.1ml/day orally of ECE of colostrum collected at zero-hour after parturition, named as (0hECE), a group that received 0.1ml/day orally of ECE of colostrum collected at 12-hour after parturition, named as (12hECE) and a control group that received only oral normal saline at 0.1ml/day, named as (NS) group. The oral treatments for all groups were lasted for 30 days. The general hematological parameters (GHPs), blood concentrations of (alkaline phosphate, creatinine, cholesterol and glucose) and serum levels of (LH, testosterone and FSH) were measured after the experiment was done.

The findings revealed that the GHPs represented by L, PCV, Hb, RBCs and WBCs were significantly ($p < 0.05$) elevated (except WBC which showed significant decreasing in the ECE-treated groups) when 0hECE or 12hECE group was compared to the NS group; however, no significant differences were noticed for 0hECE and 12hECE groups. Also, the results unveiled that creatinine, cholesterol and glucose were significantly ($p < 0.05$) decreased when 0hECE or 12hECE groups were compared to the NS group; however, no significant differences were noticed between 0hECE and 12hECE groups. The results showed that the serum levels of the LH, FSH and testosterone were significantly ($p < 0.05$) elevated in the 0hECE and 12hECE groups compared with the NS group, interestingly, significant ($p < 0.05$) differences were noticed when 0hECE and 12hECE groups were compared with each other at the levels of LH and FSH. The present work provides evidence about the beneficial effects of the ewe colostrum extract on the hematological, biochemical and hormonal parameters indicating increases in the levels of LH, FSH and testosterone in the ECE-treated groups.

Key words: Ewe colostrum, FSH, infertility, LH, testosterone.

Introduction

Different medicines have been created so far to treat male infertility and are consistently generating outcomes. Infertility is one of the most severe health and economic issues faced by developed countries. Although both cultural, such as economic advancement for females and the consequent rise in marrying age and environmental, such as the presence of pollutants and global warming, considerations are behind the increasing numbers of infertile individuals, males represent 50% of all infertility rates in population. To date, numerous methods have been created for treating male infertility, such as the use of *in-vitro* fertilizing methods (especially intracytoplasmic

sperm injection) and “TESE-ICSI” that involves the harvesting of sperms directly from the testes. Though these techniques produce constant outcomes, there was no enough demonstrated efficiency in the lack of proper viable sperms in the testes for males with nonobstructive-azoospermia (1-3).

Infertility in males relates to the failure of a male to cause conceiving. In a minimal count of one specimen of two sperm analyses obtained between one and four weeks apart, the infertility in males is understood as changes in concentration of sperms and/or their motility and/or the sperm morphology. This induces 40 to 50% of all infertility cases and 7% of all male infertility cases. Sperm

parameters under the normal WHO levels are regarded to cause infertility in males. Low levels of sperms (oligospermia), improper motility (asthenospermia) and unusual sperm structure (teratospermia) are the important factors of male infertility. Semen quantity and other seminal indicators of malfunctioning epididymis, prostate and seminal vesicles are less well linked factors with infertility in males (4-10).

Many scientists have claimed that society in well-developed countries and breaking down of living environment have led to increases in male infertility. Long-reported threatening variables involve elevated temperature in workplaces, industrial noise, radiation effects, electromagnetic fields and a wide range of chemical components have increased the infertility in males (2).

Complementary and alternative medicines (CAM) have been recognized to provide actual solutions for many health problems using different substances such as herbs or herbal extracts (11). Here, the present experimental work was focused on exploring the effects of the ewe colostrum extract (ECE) on the overall performance and the serum levels of FSH, LH and testosterone in male rats (*Rattus norvegicus*).

Materials and Methods

Ewe colostrum extract preparation

The ECE was produced from colostrum collected at zero-hour and 12-hour after parturition. Colostrum at 30ml was sterile-container-transported quickly to the Public Health Lab, Department of Public Health, College of Veterinary Medicine, University of Al-Qadisiyah, Diwaniyah, Iraq. The ewe colostrum was exposed to a centrifugation step for 10 mins at 5000rpm. Two times of a filtration step was performed on the supernatant using a microfiltration membrane, 0.22 μ m.

Animals and experimental design

The experiment was performed using 30 male rats (*Rattus norvegicus*), 10-12 weeks of age and 200gm of weight, obtained from the same mentioned college above, which were randomly separated into three groups (10 animals/group); a group that received 0.1 ml/day orally of ECE of colostrum collected at zero-hour after parturition, named as (0hECE), a group that received 0.1 ml/day orally of ECE of colostrum collected at 12-hour after parturition, named as (12hECE) and a control group that received only oral normal saline at 0.1 ml/day, named as (NS) group. The oral treatments for all groups were lasted for 30 days.

Tests

- Hematological and biochemical profiles:

Blood samples were cardiac-puncture-collected after the end of the 30 days of the treatment. The general

hematological parameters (GHPs) represented by lymphocytes (L), PCV, Hb, RBCs and WBCs were measured blood concentrations of alkaline phosphate, creatinine, cholesterol and glucose were tested. Methods used were from (12). The testosterone was measured by using coat mum count radio immune assay kit (active testosterone RIA DSL. 40 diagnostic system laboratories Inc., Texas, USA). The concentrations of luteinizing hormone (LH) and Follicle stimulating hormone (FSH) were screened by using enzyme-linked immunosorbent assay (ELISA) according to the instruction of Biochocck Inc. (Foster City, USA).

Statistical analysis

SPSS software and GraphPad Prism software v 7.00 were used to process the data and graph the processed data. Mean \pm SD was used to display the results in tables and graphs. Probability level of 5% was followed (significance was decided if p is less than this level).

Results

General hematological parameters

The findings revealed that the GHPs represented by L, PCV, Hb, RBCs and WBCs were significantly ($p < 0.05$) different (all parameters leveled up but WBCs decreased in the ECE-treated groups) when 0hECE or 12hECE group was compared to the NS group; however, no significant ($p > 0.05$) differences were noticed when 0hECE and 12hECE groups were compared with each other, table 1 and fig. 1.

For the biochemical parameters, the results unveiled that creatinine, cholesterol and glucose were significantly ($p < 0.05$) different (all parameters reduced in the ECE-treated groups) when 0hECE or 12hECE group was compared to the NS group; however, no significant ($p > 0.05$) differences were noticed when 0hECE and 12hECE groups were compared with each other, table 2 and fig. 2.

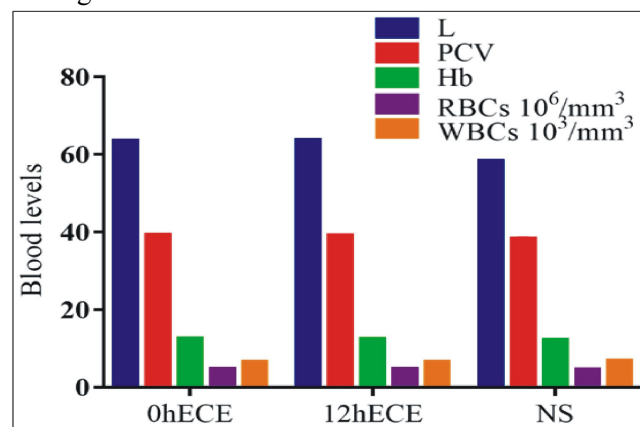


Fig. 1: General hematological parameters of the ewe colostrum extract treated groups.

Table 1: General hematological parameters of the EWE colostrum extract treated groups.

| Group | WBC ($10^3/\text{mm}^3$) | RBC ($10^6/\text{mm}^3$) | Hb | PCV | L |
|--------|-------------------------------|-------------------------------|-------------|-------------|--------------|
| 0hECE | 6.5±0.01 a | 4.55±0.04 a | 12.4±0.05 a | 39.1±0.03 a | 63.5±0.006 a |
| 12hECE | 6.4±0.01 a | 4.6±0.03 a | 12.3±0.05 a | 39.0±0.15 a | 63.6±0.003 a |
| NS | 6.7±0.03 b | 4.4±0.046 b | 12.1±0.03 b | 38.2±0.31 b | 58.3±0.06 b |

Table 2: Blood biochemical parameters of the ewe colostrum extract treated groups.

| Group | Glucose | Cholesterol | Creatinine | Alkaline phosphate IU/L |
|--------|--------------|--------------|-------------|-------------------------|
| 0hECE | 83.2±0.003 a | 91.1±0.33 a | 94.1±0.57 a | 18.61±0.005 a |
| 12hECE | 83.2±0.007 a | 91.1±0.33 a | 94.4±0.15 a | 18.61±0.01 a |
| NS | 88.1±0.005 b | 94.1±0.577 b | 94.6±0.56 b | 18.63±0.01 a |

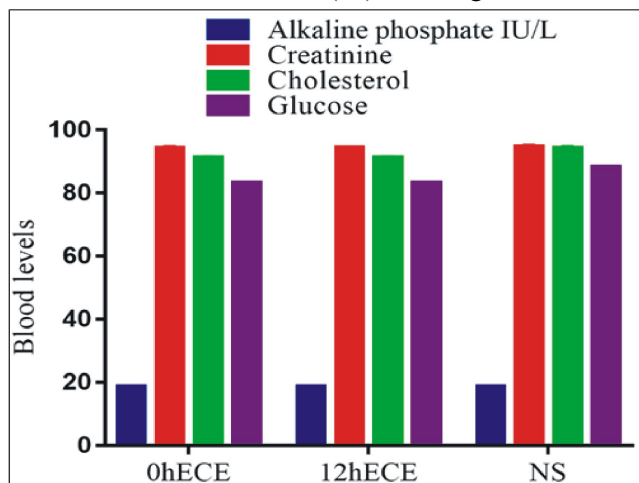
Table 3: Blood hormonal parameters of the ewe colostrum extract treated groups.

| Group | Testosterone (Mg/ml) | FSH (IU/ml) | LH (IU/ml) |
|--------|-------------------------|----------------|---------------|
| 0hECE | 1.85±1.15 a | 1.95±0.14 a | 1.43±1.62 a |
| 12hECE | 1.84±0.10 a | 1.76±0.1 b | 1.38±1.40 B |
| NS | 1.41±0.54 b | 1.19±0.056 C | 0.81±1.65 C |

For the hormonal changes, the results showed that the serum levels of the LH, FSH and testosterone were significantly ($p<0.05$) different (all parameters leveled up in the ECE-treated groups) when 0hECE or 12hECE group was compared to the NS group, interestingly, significant ($p<0.05$) differences were noticed when 0hECE and 12hECE groups were compared with each other at the levels of LH and FSH, table 3 and fig. 3.

Discussion

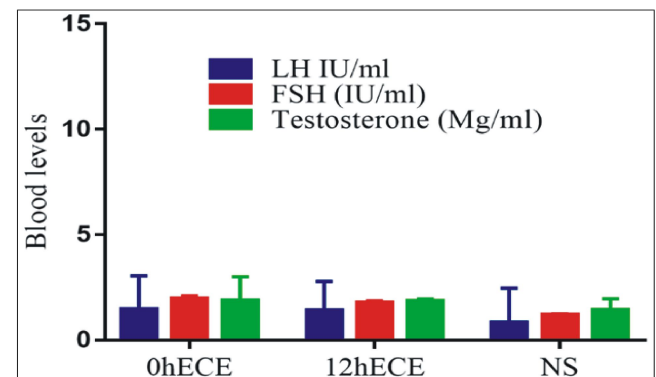
Different medicines have been created so far to treat male infertility and are consistently generating outcomes. CAMs have been recognized to provide actual solutions for many health problems using different substances such as herbs or herbal extracts (11). Although the fact that

**Fig. 2:** Blood biochemical parameters of the ewe colostrum extract treated groups.

CAMs can help in managing male infertility, thorough studies have to be accomplished for high levels of understanding the effects and mechanisms of a wide range of nutritional supplements, herbal extracts, or food materials on various health conditions that conventional medicine is facing difficulties treating them. The current investigational experiment was focused on findings effects of the EWE on biological parameters such as testosterone, FSH and LH in male rats.

For the GBPs, all parameters increased but WBCs decreased in the ECE-treated groups. It has been found that increasing blood parameters such as hemoglobin have significant effects on maintaining normal oxygenated environment in the testes and thus helps in providing better materials for spermatogenesis and preserving high quality sperms. Various sources of heme can be present; however, beef or other sources of heme-contained meat are considered as the main source for heme. The high-proliferative spermatogenesis requires high oxygen demands. In anemia, PO₂ of the testes is considerably reduced, diffusion of oxygen is minimized and thus testicular blood flow is decreased. Heme plays important roles in electron transport and enzyme catalysis and these processes are highly required by body cells and therefore the testicular cells. The malfunctioning in those processes may decrease effective spermatogenesis and also the preserving of the sperms. The current results agree with those physiological features indicating that the increases in the GHPs might have provided high quality feeding to the testicular cells in the present study rats caused by the ECE supplied (13, 14).

In the case of the blood biochemical parameters, all parameters decreased in the ECE-treated groups. The cholesterol homeostasis is important to maintain healthy

**Fig. 3:** Blood hormonal parameters of the ewe colostrum extract treated groups.

testicular cells and providing the testes with steroid precursors (15-18). The current findings, even though the cholesterol levels decreased, may indicate the proper use of this cholesterol in spermatogenesis and that is why those levels decreased in the animals treated with ECE.

For the hormonal changes, all parameters increased in the ECE-treated groups. It is well established that FSH and LH low levels due to decreases in their production have substantial effects on reducing the serum levels of testosterone which impair spermatogenesis (19). The current results showed high levels of those hormones due to the effects of ECE in the treated animals which means high quality spermatogenesis in those rats.

Conclusion

The present work provides proofs about the beneficial effects of the ewe colostrum extract on the hematological, biochemical and hormonal parameters indicating increases in the levels of LH, FSH and testosterone in the ECE-treated groups. This may provide a clear evidence of high quality spermatogenesis in the treated rats.

References

- Barnes, P.M., B. Bloom and R.L. Nahin (2008). Complementary and alternative medicine use among adults and children: United States, 2007. *Natl Health Stat Report*, [Internet]. 2008 Dec 10 [cited 2019 Aug 27]; **(12)**: 1-23. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/19361005>.
- Behre, H.M. (2019). Clinical Use of FSH in Male Infertility. *Front Endocrinol (Lausanne)*, [Internet]. 2019 [cited 2019 Aug 27]; **10**: 322. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/31178827>.
- Brugh, V.M. and L.I. Lipshultz (2004). Male factor infertility. *Med. Clin. North Am.*, [Internet]. 2004 Mar [cited 2019 Aug 27]; **88(2)**: 367-85. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/15049583>.
- Cooper, T.G., E. Noonan, S. von Eckardstein, J. Auger, H.W.G. Baker and H.M. Behre *et al.* (2010). World Health Organization reference values for human semen characteristics. *Hum Reprod Update*, [Internet]. 2010 Jan 1 [cited 2019 Aug 27]; **16(3)**: 231-45. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/19934213>.
- Coles, E.H. (1986). *Veterinary Clinical Pathology*. 4th ed. Philadelphia: Saunders.
- Cavallini, G. (2006). Male idiopathic oligoasthenoteratozoospermia. *Asian J. Androl.*, [Internet]. 2006 Mar [cited 2019 Aug 27]; **8(2)**: 143-57. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16491265>.
- Harris, I.D., C. Fronczak, L. Roth and R.B. Meacham (2011). Fertility and the aging male. *Rev. Urol.*, [Internet]. 2011 [cited 2019 Aug 27]; **13(4)**: e184-90. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22232567>.
- Hirsh, A. (2003). Male subfertility. *B.M.J.* [Internet]. 2003 Sep 20 [cited 2019 Aug 27]; **327(7416)**: 669-72. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/14500443>.
- Kumar, N. and A.K. Singh (2015). Trends of male factor infertility, an important cause of infertility: A review of literature. *J. Hum. Reprod. Sci.*, [Internet]. 2015 [cited 2019 Aug 27]; **8(4)**: 191-6. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/26752853>.
- Lotti, F. and M. Maggi (2015). Ultrasound of the male genital tract in relation to male reproductive health. *Hum Reprod Update*, [Internet]. 2015 Jan 1 [cited 2019 Aug 27]; **21(1)**: 56-83. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/25038770>.
- Leaver, R.B. (2016). Male infertility: an overview of causes and treatment options. *Br. J. Nurs.*, [Internet]. 2016 Oct 13 [cited 2019 Aug 27]; **25(18)**: S35-40. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/27734725>.
- Matzuk, M.M. and D.J. Lamb (2002). Genetic dissection of mammalian fertility pathways. *Nat Med.*, [Internet]. 2002 Oct 1 [cited 2019 Aug 27]; **8(S10)**: S40-S40. Available from: <http://www.nature.com/articles/nm-fertilityS41>.
- Miyamoto, T., A. Tsujimura, Y. Miyagawa, E. Koh, M. Namiki and K. Sengoku (2012). Male infertility and its causes in human. *Adv. Urol.*, [Internet]. 2012 [cited 2019 Aug 27]; **2012**: 384520. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22046184>.
- Plachot, M., J. Belaisch-Allart, J.M. Mayenga, A. Chouraqi, L. Tesquier and A.M. Serkine (2002). Outcome of conventional IVF and ICSI on sibling oocytes in mild male factor infertility. *Hum. Reprod.*, [Internet]. 2002 Feb 1 [cited 2019 Aug 27]; **17(2)**: 362-9. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/11821279>.
- Parton, R. and J.F. Hancock (2004). Lipid rafts and plasma membrane microorganization: insights from Ras. *Trends Cell. Biol.*, [Internet]. 2004 Mar [cited 2019 Aug 27]; **14(3)**: 141-7. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/15003623>.
- Sèdes, L., L. Thirouard, S. Maqdasy, M. Garcia, F. Caira and J.M.A. Lobaccaro *et al.* (2018). Cholesterol: A Gatekeeper of Male Fertility? *Front Endocrinol (Lausanne)*, [Internet]. 2018 [cited 2019 Aug 27]; **9**: 369. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/30072948>.
- Tvrda, E., R. Peer, S.C. Sikka and A. Agarwal (2015). Iron and copper in male reproduction: a double-edged sword. *J. Assist. Reprod Genet.*, [Internet]. 2015 Jan [cited 2019 Aug 27]; **32(1)**: 3-16. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/25245929>.
- Yokoyama, S. (2000). Release of cellular cholesterol: molecular mechanism for cholesterol homeostasis in cells and in the body. *Biochim Biophys Acta*, [Internet]. 2000 Dec 15 [cited 2019 Aug 27]; **1529(1-3)**: 231-44. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/11111092>.
- Zhou, S.J., M.J. Schilling and M. Makrides (2005). Evaluation of an iron specific checklist for the assessment of dietary iron intake in pregnant and postpartum women. *Nutrition*, [Internet]. 2005 Sep [cited 2019 Aug 27]; **21(9)**: 908-13. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16039831>.