Effect of *Punica granatum, Citrus limon* and their combinations on the plasma Gonadotropins in female rabbits

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Abstract: Fruits produce revitalizing effects, hence the impact of *Punica granatum, Citrus limon* and their combinations have been investigated on the plasma levels of gonadotropin, testosterone and sexual development capacity in female rabbits. Ninety female rabbits were randomly assigned into nine groups, each comprising of ten animals. One group was given saline and designated as control. Three groups were given *P. granatum* 2mL /kg, 5mL/kg, 8mL/kg, other three groups received *C. limon* 0.2mL/kg, 0.4mL/kg, 0.6mL/kg respectively, remaining groups received *C. limon* and *P. granatum* in combination i.e. 0.4mL/kg *C. limon* + 5mL/kg *P. granatum* and 0.2mL/kg *C. limon* + 8mL/kg *P. granatum*. Juices were administered once daily by mouth from day 0 of pups delivered to postnatal day15. Blood samples were gathered from ear vein at day11 and day15. There was significant increase in follicle stimulating hormone by *P. granatum* at 5 and 8mL/kg on day 11 and 15, by *C. limo*n at 0.4 and 0.6mL/kg on day11, 0.4mL/kg at day15, by combination doses of *C. limon* and *P. granatum* 0.4 +5mL/kg at day 11, 0.4+5 mL/kg and 0.2 + 8mL/kg at day15. There was also significant increase in luteinizing hormone by *P. granatum* at 2, 5 and 8mL/kg and by *C. limo*n 0.4mL/kg at day11. There was highly significant increase on day 11 in LH at combination doses of *C. limon* and *P. granatum* 0.4 + 5mL/kg. There was significant increase in testosterone level by *P. granatum* at 2, 5 and 8mL/kg on day 11 and 5mL/kg on day15 and highly significant increase at 2 and 8mL/kg. *C. limo*n caused significant increase in testosterone at 0.4 mL/kg on day11, 0.2 and 0.6mL/kg on day 15 and highly significant increase at 0.4mL/kg on day15. Whereas combinations doses of *C. limon* and *P. granatum* at 0.4+5mL/kg caused highly significant increase in testosterone level as compare to control. Results of present study revealed increase in plasma gonadotropin and testosterone levels showing increase in sexual capacity of female rabbits which could be mainly accounted for high vitamin C and flavonoids contents of these juices.

Keywords: *Citrus limon*, Follicle stimulating hormone, Luteinizing hormone, *Punica granatum*, Testosterone.

INTRODUCTION

Follicle stimulating hormone (FSH) plays an important role to retain the normal function of the ovary in producing oocytes and hormones in all mammalian species. When there is lack of FSH, follicular growth is detained and female becomes infertile (Findlay and Drummond, 1999). Luteinizing hormone (LH) is the key supervisor for the functions of leydig cells and studies suggest that FSH can affect leydig cell indirectly through sertoli cell derived factors (Sriraman and Rao, 2004). Many factors have been suggested to be the mediators among these two cell types such as cytokines, growth factors and steroids (Saez and Lejeune, 1996). Testosterone (TS) is well known for its actions in reproduction as well as in the regulation of emotionality, reinforcement and memory processes (Buddenberg *et al*., 2009; Topic *et al*., 2007).

The ultimate goal of infertility therapy is to achieve pregnancy in the harmless, effective and suitable way, using the highest purity agent's available (Latash *et al*., 2004). Though recent technology is rapidly developing recombinant LH and FSH preparation yet, there is a need to develop oral and transdermal gonadotropin agonists to eradicate the use of injections.

Phytoestrogens i.e. flavonoids, synthesized by plants and mostly found in fruits are associated with the cure of specific diseases of the reproductive organs (Pedersen *et al*., 2000; Gutendorf and Westendorf, 2001; Wang *et al*., 2002). They have a significant role as suppressor of free radicals and act as antioxidants (Grassi *et al*., 2010; Lee *et al*., 2016). Hence alterations in synthesis of Reactive oxygen species (ROS) in gonads stimulate the oxidation and DNA damage of cells (Sikka, 1996; de Lamirande *et al*., 1997; Sanocka and Kurpisz, 2004; Henkel, 2005).

P. granatum contains substantial amounts of flavonoids such as quercetin, kaempferol, ellagitannins, gallotannins and anthocyanins (Fischer *et al*., 2011). Moreover presence of minerals, electrolytes and vitamin C increases its nutritional activity (Turk *et al*., 2008; Mirdeghan *et al*., 2007). Testosterone, estrogen and estradiol in *P. granatum* make it useful in variety of ailments (Gil *et al*., 2000; Langley, 2000; Lansky and Newman, 2007).

Flavonoids found in *C. limon* includes rutin, hesperidin, quercitrin, eriocitrin, narirutin, didymin and naringin

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(Tripoli *et al*., 2007; Nijveldt *et al*., 2001). Role of naringin have been stated for its antidiabetic effects (Pari and Suman 2010). It is well known that vitamin C, folate, carotenoid and phenolic contents abundant in *C. limon* are beneficial to human health and is described to be the source of magnesium, potassium, folic acid, limonoids and xanthoxyletin (Deyhim *et al*., 2006). Number of studies has suggested its antioxidant, hypocholesterolemic, antifungal, anthelminthic, anticancer, anti-inflammatory, gastro protective and anti-microbial effects (Dhanavade *et al*., 2011; Chun *et al.,* 2008; Viuda *et al*., 2008; Rozza *et al*., 2011).

Numerous combination studies of fruit juices, reported their synergistic effect in many system of health (Riaz *et al*., 2010; Riaz *et al*., 2014). Thus, in presence of various evidences regarding use of fruit juice as dietary substances current study was planned to examine the hormonal effect of *C. limon*, *P. granatum* and their combinations on gonadotropins, testosterone and sexual behavior in female rabbits.

MATERIALS AND METHODS

Animals

Ninety healthy white female rabbits with mean body weight of 1300±50grams were randomly distributed in different groups each comprising of ten rabbits. Individual rabbits were accommodated in stain less steel cages, under particular condition of temperature 23±2°C and humidity 50-60%. Animals were preserved during the experiment on a 12/12h light and dark cycle with free contact to rabbit chow and tap water. The use of animals during the study was in accordance with the National Institute of Health (NIH) guide for the care and use of Laboratory Animals (Council, 1996) and permitted by the Board of Advance Studies and Research University of Karachi.

Administration of Juices

Citrus Limon

C. limon was procured from local market and recognized by plant conservation center, University of Karachi. The coupon specimen no C.L 11-11 was placed in department of Pharmacognosy, University of Karachi. Fresh juice of *C. limon* was obtained by pressing the fruit by hand which was filtered and administered in three doses i.e. 0.2, 0.4 and 0.6ml/kg according to body weight through oral route and was considered as CL-1, CL-2 and CL-3 respectively.

Punica granatum

P. granatum was procured from local market, recognized by plant conservation center, University of Karachi. The coupon specimen no P.G 11-12 was placed in department of Pharmacognosy, University of Karachi. Fresh juice of; *P. granatum* was obtained by pressing the fruit by hand which was filtered and administered in three doses i.e. 2, 5 and 8ml/kg according to body weight through oral route and was considered as PG-1, PG-2 and PG-3 respectively.

Combinations of C. Limon and P. granatum

C. limon and *P. granatum* were given orally in two combination doses i.e. 0.4ml/kg CL+5ml/kg PG and 0.2ml/kg CL+8ml/kg PG and was abbreviated as CP-1 (0.4+5ml/kg) and CP-2 (0.2+8ml/kg). All doses were given once daily through oral route.

Design of experiment

Female rabbits were kept with male rabbits until pregnancy was induced. Ninety female rabbits were distributed into nine groups, each containing ten animals. Control group was given normal saline, three groups were given *C. limon* and designated as CL-1, CL-2, CL-3, three groups on *P. granatum* were labeled as PG-1, PG-2, PG-3 and two groups received combination doses of CL and PG mentioned as CP-1, CP-2. This study was scheduled for fifteen days. Dosing was started once daily orally, from day of pups birth designated as postnatal day0 and continued up to the postnatal day15 (day15). Entire study was completed under NCCL guideline (Wayne, 1998). Blood samples were gathered from ear vein in gel tubes at day11 and day15 after completion of dosing period.

Determination of FSH, LH and TS

Serum FSH, LH and TS levels were determined using Elisa kits (Human, Germany) as per manufacturer instructions. The activity levels of FSH, LH and TS in the samples were measured photometrically and their intensity of color was directly proportional to concentration in ng/ml.

STATISTICAL ANALYSIS

Data was entered and analyzed using by SPSS version 17 and represented as mean ± S.E.M with 95% confidence interval. ANOVA followed by post hoc was used for comparisons of values with control. Values of $p \leq 0.05$ were considered significant and $p \leq 0.005$ as highly significant.

RESULTS

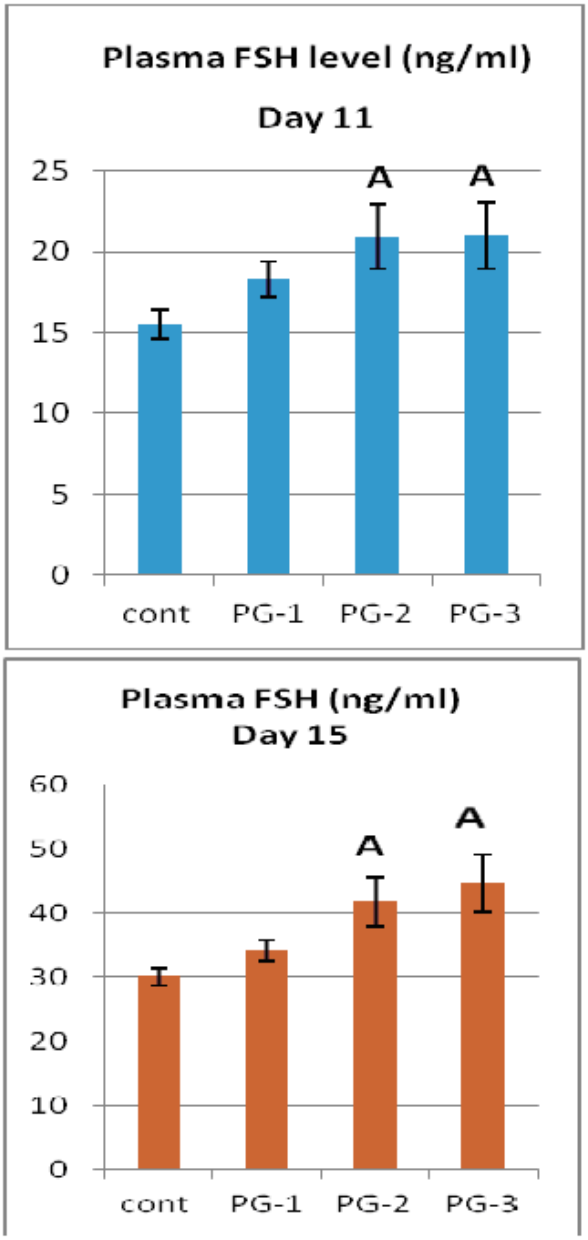
Effect on FSH

Fig. 1 reveals the effect of *P. granatum* on FSH. There was significant increase in FSH level in both animal groups of *P. granatum* i.e. PG-2 and PG-3at day 11 and day 15, as related to control animals.

Fig. 2 reveals the effect of *C. limon* on FSH. Significant increase in FSH was observed by both CL-2 and CL-3, however increase by CL-2 was on day 11 and 15, but increase by CL-3 was only on day 11 as compare to control.

Fig. 3 reveals the effect of combination doses of CL and PG on FSH. Significant increase in FSH was observed by CP-1 on both days 11 and 15 as compare to control,

whereas CP-2 resulted in significant increase in FSH on day 15 only as compare to control.



n=10, Average values ± S.E.M. Columns with (A) differ significantly, P ≤ 0.05 related to control, cont =control; PG-1=*P. granatum* 2 mL/kg; PG-2= *P. granatum* 5 mL/kg; PG-3= *P. granatum* 8 mL/kg

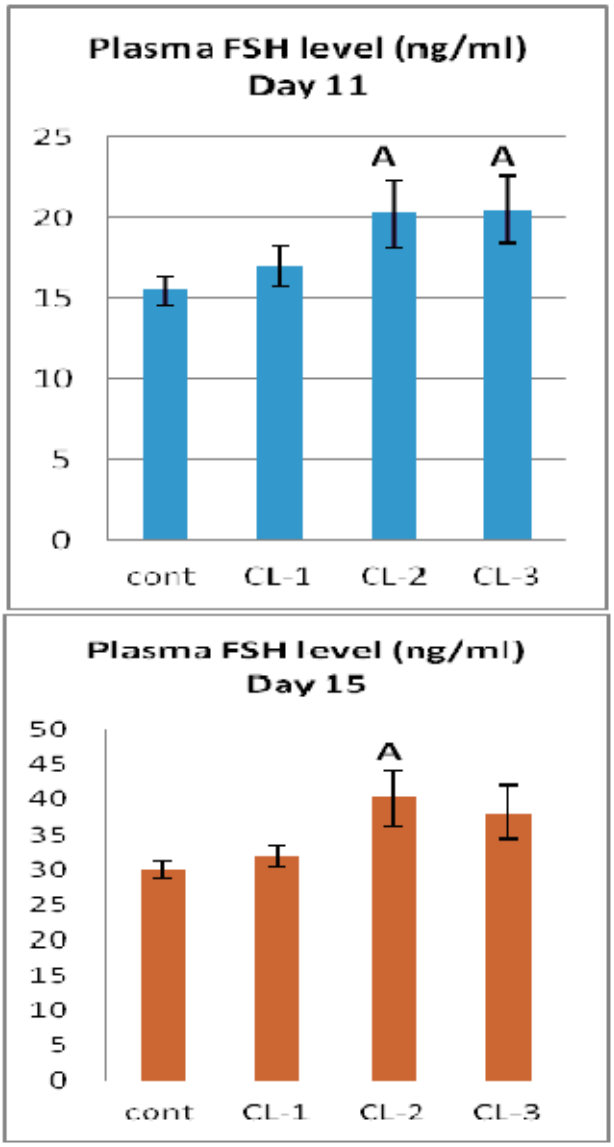
Fig. 1: Effect of *P. granatum* on FSH

Effect on LH

Fig. 4 reveals the effect of PG on luteinizing hormone (LH). Significant increase in LH was observed by all three doses at day 11 as compared to control.

Fig. 5 shows the effect of CL on LH. There was significant increase in LH at day 11 by CL-2 as compare to control.

Fig. 6 reveals the effect of combination doses of CL and PG on LH. Highly significant increase in LH was observed by CP-1 on day 11 only, while no significant change was observed by CP-2 as compare to control.



n=10, Average values ± S.E.M. Columns with (A) differ significantly, P ≤ 0.05 related to control cont =control; CL-1=*C. limon* 0.2mL/kg; CL-2= *C. limon* 0.4mL/kg; CL-3= *C. limon* 0.6mL/kg

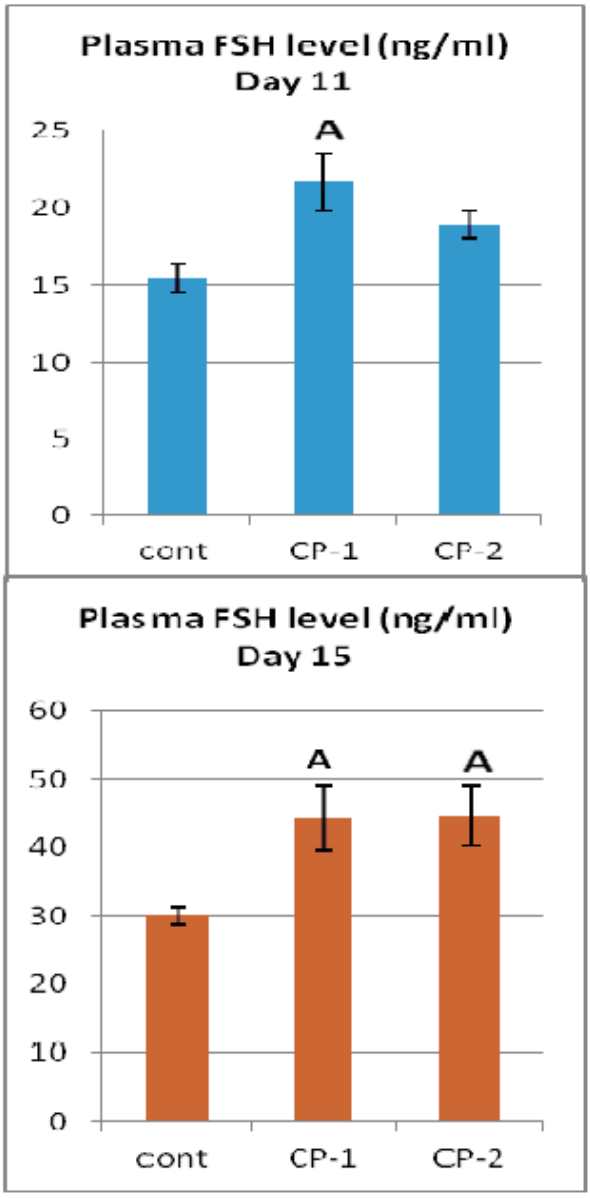
Fig. 2: Effect of *C. limon* on FSH

Effect on TS

Fig. 7 shows the effect of PG on testosterone level. Significant increase in testosterone level on day 11 was observed at all three doses as compared to control. However on day 15 there was highly significant rise in testosterone by PG-1 and PG-3 and significant increase by PG-2 as compare to control.

Fig. 8 reveals the effect of CL on testosterone. There was

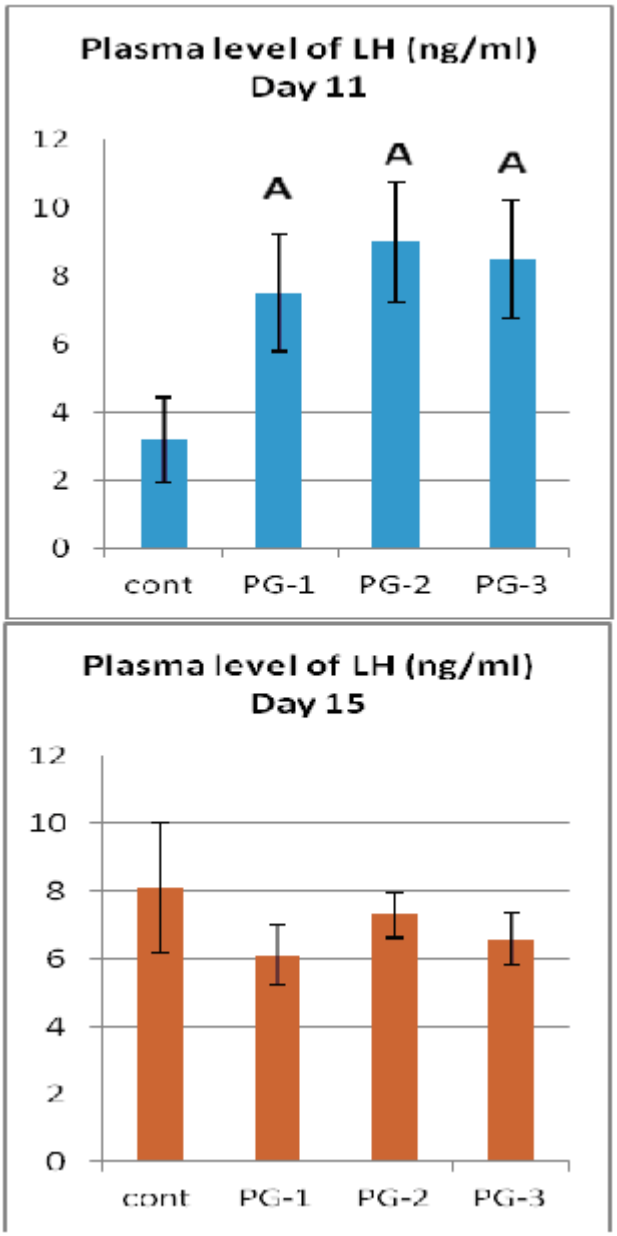
significant increase in testosterone level by CL-2 on day 11 and highly significant increase on day 15 as compare to control, while at day 15 testosterone level was significantly increased by CL-1 and CL-3 as compare to control animals.



n=10, Average values ± S.E.M. Columns with (A) differ significantly, P≤0.05 related to control cont=control; CP-1= 0.4 mL/kg *C. limon* +5 mL/kg *P. granatum*; CP-2= 0.2mL/kg *C. limon*+ 8mL/kg *P. granatum*

Fig. 3: Effect of combination doses of *C. limon* and *P. granatum* on FSH

Fig. 9 reveals the effect of combination doses of CL and PG on testosterone. There was highly significant rise in testosterone by CP-1 and CP-2 on day 11. While at day 15 there was highly significant rise in testosterone by CP-1 and significant increase by CP-2 as compare to control.



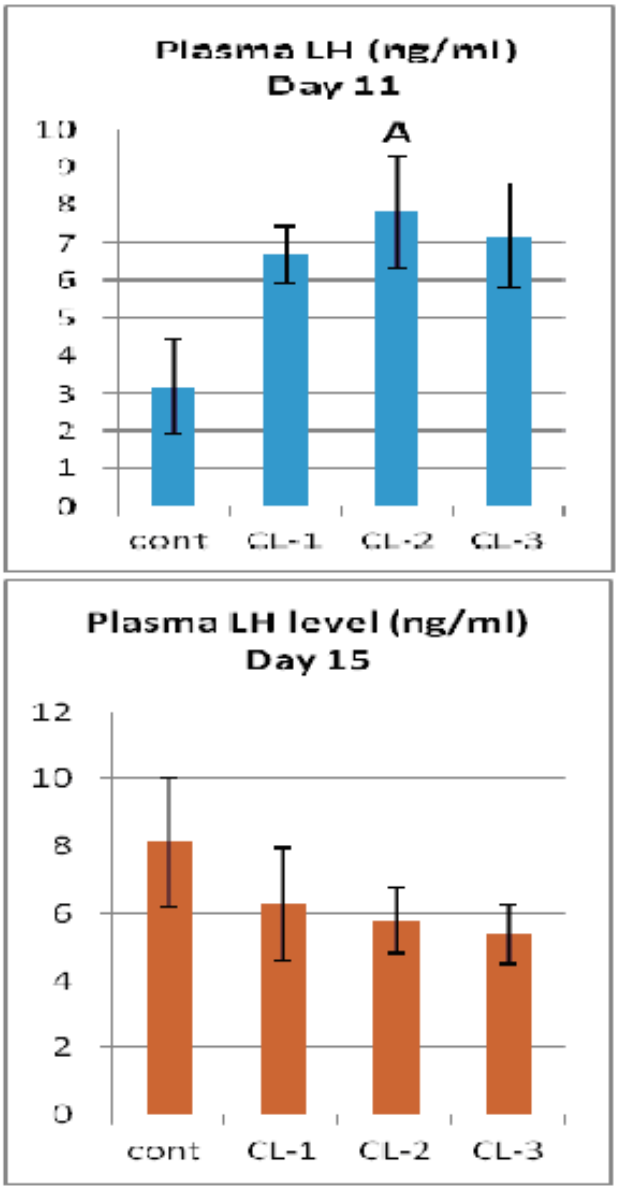
n=10, Average values ± S.E.M. Columns with (A) differ significantly, P ≤ 0.05 related to control

Fig. 4: Effect of *P. granatum* on LH

DISCUSSION

Flavonoids are naturally occurring molecules abundantly found in fruit and vegetables, number of studies have suggested an inverse association between flavonoids intake and various disorders, due to their antioxidant properties. Since flavonoids as antioxidant remove and prevent the formation of ROS and lipid per-oxidation. Hence alterations in synthesis of ROS in gonads kindle the oxidation and DNA damage of cells (Sanocka and Kurpisz, 2004; Henkel, 2005; Grassi *et al*., 2010). Present study specifically evaluates the therapeutic effectiveness

of flavonoids rich fruits *C. limon* and *P. granatum* along with their combinations (CP) on plasma gonadotropins.

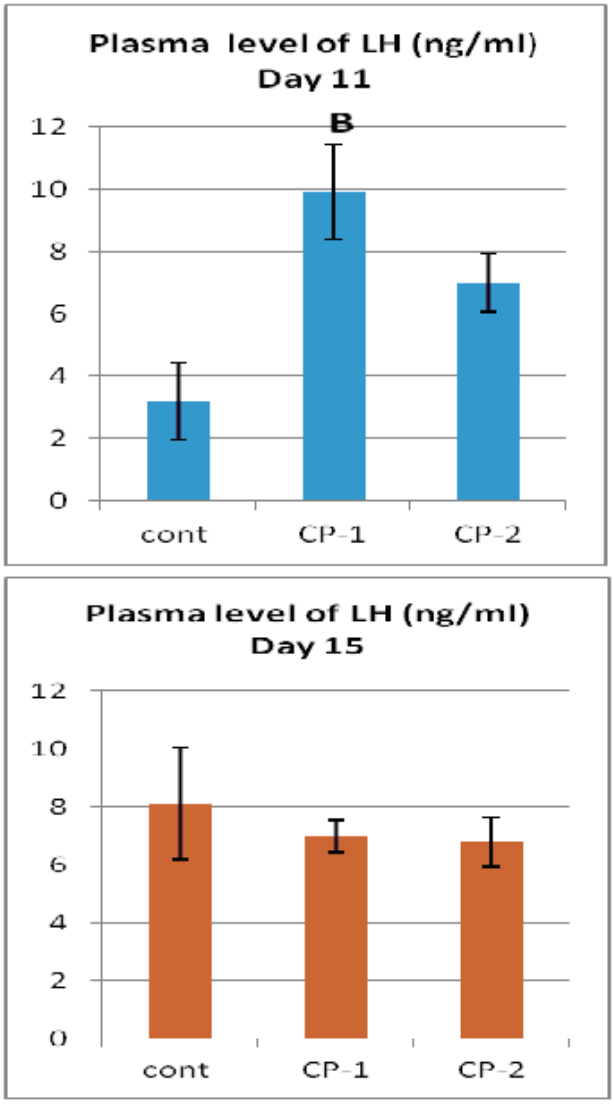


n=10, Average values ± S.E.M. Columns with (A) differ significantly, $P \leq 0.05$ related to control

Fig. 5: Effect of *Citrus limon* on LH

In present study rabbits were selected, since biochemical and histopathological changes produced in rabbits are comparatively similar to humans (Irena *et al*., 1979). Moreover sufficient amount of blood samples can easily be obtained twice at Post natal days (PND) 11 and 15. These treatment paradigms were chosen so as to collect blood during the rise (day11) and during the peak (day15) of plasma FSH in female rabbits (Wilson *et al.,* 1998).

PG is a vital source of anthocyanins, hydrolysable tannins punicalagin and punicalin, ellagic and gallic acids and vitamin C. Their antioxidant and free radical searching activity have been reported. Results of present study showed significant increase in plasma FSH and testosterone level by PG, this might be due to the presence of testosterone and estrogen like compounds in PG (Kim *et al*., 2004; Lansky *et al*., 2005). Dihydrotestosterone (DHT) can excite FSH secretion in vitro responsible for infantile surge of FSH and its responsiveness to GnRH even if there is ovariectomy (Tohei and Kogo 1999). Report of Wilson *et al*., (1998) showed relationship of estrogen with FSH. According to this report estrogen receptor antagonists can suppress FSH secretion.

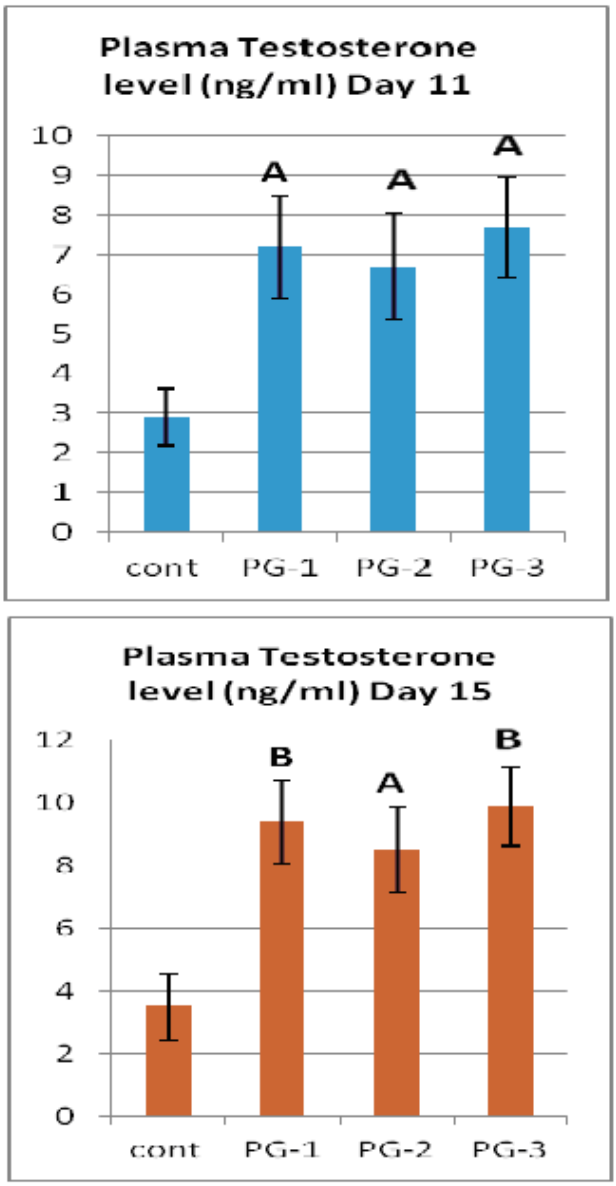


n=10, Average values ± S.E.M. Columns with (B) differ highly significantly, $P \leq 0.005$ related to control

Fig. 6: Effect of combination doses of *C. limon* and *P. granatum* on LH

Result of present study also showed significant increase of serum FSH and TS by CL. This might be due to abundant flavonoids present in CL, which are reported to influence all steroidal function by increase in protein synthesis via cAMP-dependent protein kinase A (PKA)

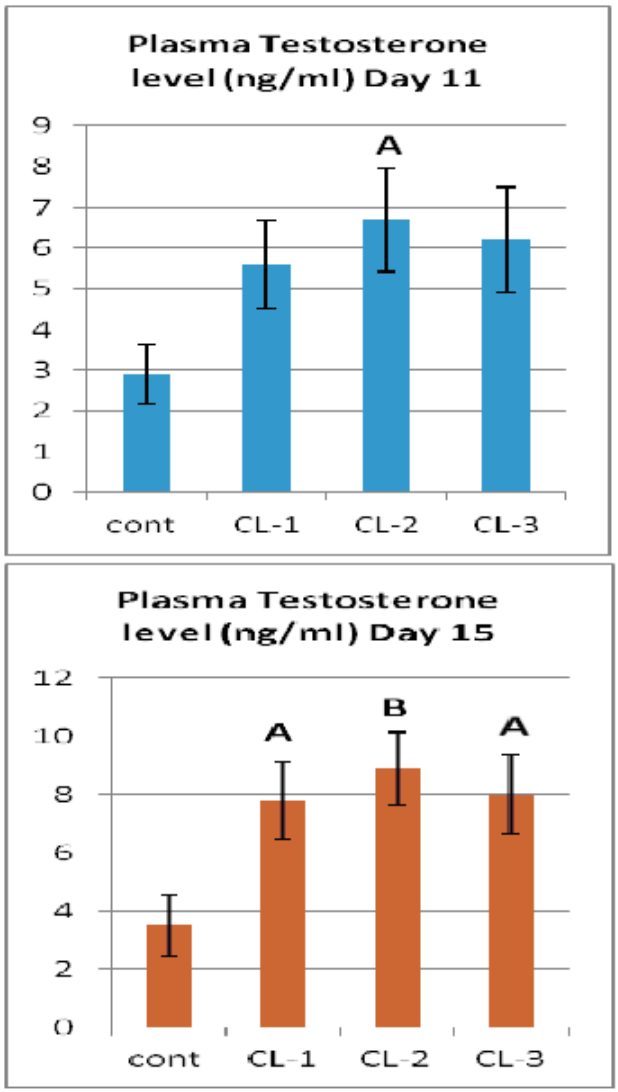
pathway, since interaction of FSH with its receptors activates adenylyl cyclase (AC) that leads to the production of cAMP, which in turn activates PKA (Yu *et al*., 2003). Another possible mechanism by which serum FSH and TS level may increase is probably the result of the loss of direct central nervous inhibition of FSH secretion by GnRH, that promotes the synthesis and secretion of FSH and LH in female rabbits and speeds up the development of ovary and follicles in female rabbits (Wi *et al*., 2012; Kerr and Sharpe, 2013).



n=10, Average values ± S.E.M. Columns with (A) differ significantly, P≤0.05 as related to control Columns with (B) differ highly significantly, P≤0.005 as related to control

Fig. 7: Effect of *P. granatum* on Testosterone

Results of present study also demonstrated significant increase in FSH and TS by CP-1 almost similar to PG and CL. Hence it could be postulated that estrogen like components in PG and flavonoids in CL have activates steroidal receptors, giving estrogen like effect (Wilcox *et al*., 1999; Kim *et al*., 2002) are by CP-1 on FSH and TS. Progesterone in PG has been shown to modulate levels of FSH, signifying a role for progesterone in the regulation of FSH synthesis, hence it may be suggested that alterations in gonadotropins secretion of the female rabbits during sexual development is due to these essential components (Lansky and Newman, 2007). Thus PG is used as a natural symbol of fertility in many cultures (Mahdihassan, 1984; Fawole and Opara., 2013).

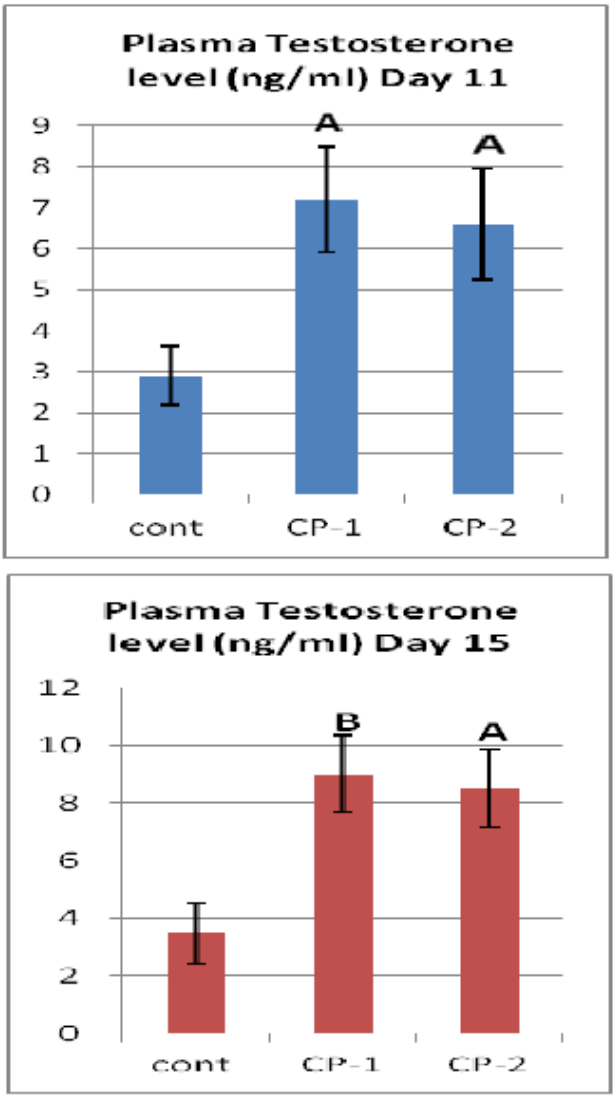


n=10, Average values ± S.E.M. Columns with (A) differ significantly, P ≤ 0.05 related to control Columns with (B) differ highly significant, P ≤ 0.005 related to control

Fig. 8: Effect of *C. limon* on Testosterone

Results of current study revealed significant increase in LH by PG, CL and CP at PND 11, which is consistent with the result of Wilson and Handa (1997) who revealed that LH was inhibited by GnRH antagonist at PND 11, suggesting the role of progesterone as GnRH agonist

increasing level of LH (Lansky and Newman, 2007). While result observed at PND 15 in present study by PG, CL and CP matches with the report of Wilson and Handa (1998). According to this report no significant change was observed at PND 15, indirectly support our results and suggest that LH secretion altered in treated animals from PND 12-15.



n=10, Average values ± S.E.M. Columns with (A) differ significantly, $P \leq 0.05$ related to control Columns with (B) differ highly significantly, $P \leq 0.005$ related to control

Fig. 9: Effect of combination doses of *C. limon* and *P. granatum* on Testosterone

No significant effect of these juices on LH secretion at PND 15 may be due to variable level of LH secretion at this time and this fluctuation was also observed in the control animals as well. The high variable level of LH during the infantile period is thought to be caused by an immature regulatory system of LH by GnRH and result in variable burst of LH. This data indicates that the cells of the infantile pituitary contain functional testosterone and estrogen receptors that are capable of responding to steroid, or steroid like compounds (O'Conner *et al.,* 1999; Tohei and Kogo, 1999).

Report of Cho *et al*., (2013) suggests that antioxidant action of flavonoids affect gonadotropins. They modulate antioxidant enzyme activity or interrupt signaling cascade and plays important role in blocking oxidative hormonal injury. Therefore it may be suggested that flavonoids richly present in *C. limon* and *P. granatum* may be responsible for favorable effect of these fruits on gonadotropins.

Overall data suggests that testosterone and estrogen like compounds in these fruits act on FSH and LH in early infantile period in a positive fashion (Sreeja *et al*., 2012; choi *et al*., 2006). This increase in FSH and LH level may also be due to chloride presence in these fruits (Waheed *et al*., 2004; Gonzalez *et al*., 2010), since there are several studies which demonstrates the possible role of chloride in the regulation of both FSH and LH (Dutt *et al*., 1978; Ramalingam *et al*., 2003). Hence it may be concluded that concomitant administration of PG, CL and CP to female rabbits improves the reduced fertility. Therefore, it seems logical to advise infertile patients to include PG and CL juices in their diet to minimize or prevent the female fertility and avoid adverse effect of drugs taken for this purpose.

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REFERENCES

Buddenberg TE, Komorowski M, Ruoccob LA, de Souza Silva MA and Topic B (2009). Attenuating effects of testosterone on depressive-like behavior in the forced swim test in healthy male rats. *Brain Research Bulletin,* **79**: 182-186.

Cho N, Lee KY, Huh J, Choi JH, Yang H, Jeong EJ, Kim HP and Sung SH (2013). Cognitive-enhancing effects of Rhusverniciflua bark extract and its active flavonoids with neuroprotective and anti-inflammatory activities. *Food Chem. Toxicol.,* **58**: 355-361.

Choi DW, Kim JY, Choi SH, Jung HS, Kim HJ, Cho SY, Kang CS and Chang SY (2006). Identification of steroid hormones in pomegranate (Punica granatum) using HPLC and GC-mass spectrometry. *Food Chem.,* **96**: 562-571.

Chun OK, Chung S-Jin, Claycombe KJ and Song WO (2008). Serum C-reactive protein concentrations are inversely associated with dietary flavonoid intake in U.S. adults. *J. Nutr.,* **138**: 753-760.

Council NR (1996) Guide for the care and use of laboratory animals. National Academy Press, Washington, pp.1-7.

Dhanavade MJ, Jalkute CB, Ghosh JS and Sonawane KD (2011). Study Antimicrobial Activity of Lemon (Citrus lemon L.) Peel Extract. *Br. J. Pharmacol. Toxicol.,* **2**: 119-122.

de Lamirande E, Jiang H, Zini A, Kodama H and Gagnon C (1997). Reactive oxygen species and sperm physiology. *Rev. Reprod.,* **2**: 48-54.

Deyhim F, Garica K, Lopez E, Gonzalez J, Ino S, Garcia M and Patil BS (2006). Citrus juice modulates bone strength in male senescent rat model of osteoporosis. *Nutrition*, **22**: 559-563.

Dutt GNH, Yashi WK and Inoue S (1978). Cadmium chloride on the serum level of follicle stimulating hormone (FSH), leutinizing hormone (LH) and androgen in the adult male rat. *Proc. Indian Acad.,* **87**: 161-167.

Fawole OA and Opara LU (2013). Developmental changes in maturity indices of pomegranate fruit: A descriptive review. *Scientia Horticulturae.,* **159**: 152-161.

Findlay JK and Drummond AE (1999). Regulation of the FSH Receptor in the Ovary. *Trends Endocrinol. Metab.,* **10**: 183-188.

Fischer UA, Carle R and Kammerer DR (2011). Identification and quantification of phenolic compounds from pomegranate (Punica granatum L.) peel, mesocarp, aril and differently produced juices by HPLC-DAD-ESI/MSn. Food Chemistry **127**: 807-821.

Gil MI, Tomas-Barberan FA, Hess-Pierce B, Holcroft DM and Kader AA (2000). Antioxidant Activity of Pomegranate Juice and Its Relationship with Phenolic Composition and Processing. *J. Agric. Food Chem.,* **48**: 4581-4589

Gonzalez-Molina E, Domínguez-Perles R, Moreno DA and García-Viguera C (2010). Natural bioactive compounds of Citrus limon for food and health. *J Pharm. Biomed. Anal.,* **51**: 327-345.

Grassi D, Desideri G and Ferri C (2010). Flavonoids: Antioxidants against Atherosclerosis. *Nutrients,* **2**: 889-902.

Gutendorf B and Westendorf J (2001). Comparison of an array of in vitro assays for the assessment of the estrogenic potential of natural and synthetic estrogens, phytoestrogens and xenoestrogens. *Toxicol.,* **166**: 79-89.

Henkel R (2005). The impact of oxidants on sperm functions. *Andrologia.,* **37**: 205-206.

Irena C, Kazimier K, Leopold Andreze JK, Krystyna S (1979). Blood serum protein in experimental chronic liver injury in rabbits. *Pathol. Pol.,* **30**: 71-88

Kerr JB and Sharpe RM (2013). Follicle-Stimulating Hormone Induction of Ley dig Cell Maturation. DOI: http://dx.doi.org/10.1210/endo-116-6-2592.

Kim HP, Son KH, Chang HW and Kang SS (2004). Anti-inflammatory Plant Flavonoids and Cellular Action Mechanisms. *J. Pharm. Sci.,* **96**: 229-245.

Lansky EP, Jiang W, Mo H, Bravo L, Froom P, Yu W, Harris NM, Neeman I and Campbell MJ (2005). Possible synergistic prostate cancer suppression by anatomically discrete pomegranate fractions. *Investigational New Drugs,* **23**: 11-20.

Lansky EP and Newman RA (2007). Punica granatum (pomegranate) and its potential for prevention and treatment of inflammation and cancer. *J. Ethnopharmacol.,* **109**: 177-206.

Latash WD, Karande VC and Klipstein S (2004). The development of recombinant follicle stimulating hormone (rFSH) for use in the treatment of infertility: new solutions to old challenges. Reviews in Gynaecological Practice **4**: 203-210.

Lee LS, Choi EJ, Kim CH, Sung JM, Kim YB. Dong-Ho S, Choi HW, Choi YS, Kum JS and Park JD (2016). Contribution of flavonoids to the antioxidant properties of common and tartary buckwheat. *Jour Cereal Sci.,* **68**: 181-186.

Mahdihassan S (1984). Outline of the beginning of alchemy and its antecedents. *Am. J. Chin. Med.,* **12**: 32-42.

Mirdeghan SH and Rehemi M (2007). Seasonal changes of mineral nutrients and phenolics in pomegranate. *Scientia Horticulturae.,* **111**: 120-127.

Nijveldt R, Nood E.van, Hoorn DEC van, Boelens PG, Norren K.van and Leeuwen PAM van (2001). Flavonoids: a review of probable mechanisms of action and potential applications. *Am. J. Clin. Nutr.,* **74**: 418-425.

O'Conner JL, Wade MF, Edwards DP and Mahesh VB (1999). Progesterone and regulation of the follicle-stimulating hormone (FSH-β) gene. Steroids. **64**: 592-597.

Pari L and Suman S (2010). Antihyperglycemic and antilipidperoxidative effect of flavonoid naringin in streptozotocin-nictonamide induced diabetic rats. *Int. J. Biol. Med. Res.,* **1**: 206-210.

Pedersen CB, Kyle J, Jenkinson AM, Gardner PT, McPhail DB and Duthie GG (2000). Effects of blueberry and cranberry juice consumption on the plasma antioxidant capacity of healthy female volunteers. *Eur. J. Clin. Nutr.,* **54**: 405-408.

Ramalingam V, Vimaladevi V, Rajeswary S and Suryavathi V (2003). Effect of mercuric chloride on circulating hormones in adult albino rats. *J. Environ Biol.,* **24**: 401- 404.

Riaz A, Khan RA, Ahmed S and Afroz S (2010). Assessment of acute toxicity and reproductive capability of a herbal combination. *Pak. J. Pharm. Sci.,* **23**: 291-294.

Riaz A, Khan RA, Algahtani HA (2014). Memory boosting effect of Citrus limon, Pomegranate and their combinations. *Pak. J. Pharm. Sci.,* **27**: 1837-1840.

Rozza AL, Moraesb de Mello T, Kushimab H, Tanimotoa A, Marquesc Mom, Bauabd TM, Hiruma-Limab CA, Pellizzona CH (2011). Gastroprotective mechanisms of Citrus lemon (Rutaceae) essential oil and its majority compounds limonene and _-pinene: Involvement of heat-shock protein-70, vasoactive intestinal peptide, glutathione, sulfhydryl compounds, nitric oxide and prostaglandin E2 Chemico-Biological Interactions, **189**: 82-89.

Saez JM and Lejeune H (1996). Regulation of Leydig cell functions by hormones and growth factors other than LH and IGF-1. In: Payne, A.H., Hardy, M.P., Russell, L.D. (Eds.), The Leydig Cell. Cache River Press, Vienna, IL, pp.383-406.

Sanocka D and Kurpisz M (2004). Reactive oxygen species and sperm cells. *Reprod. Biol. Endocrinol.,* **2**: 1-7.

Sikka SC (1996). Oxidative stress and role of antioxidants in normal and abnormal sperm function. *Front Biosci.,* **1**: 78-86.

Sreeja S, Kumar TRS, Lakshmi BS and Sreej S (2012). Pomegranate extract demonstrate a selective estrogen receptor modulator profile in human tumor cell lines and *in vivo* models of estrogen deprivation. *J. Nutrit. Biochem.,* **23**: 725-732.

Sriraman V and Rao AJ (2004). Evaluation of the role of FSH in regulation of Leydig cell function during different stages of its differentiation. *Mole. Cell Endocrinol.,* **224**: 73-82.

Tohei A and Kogo H (1999). Dexamethasone increases follicle-stimulating hormone secretion via suppression of inhibin in rats. *Euro J. Pharmacol.,* **386**: 69-74.

Topic B, Tavares MC, Tomaz C and Mattern C (2007) Prolonged effects of intra-nasally administered testosterone on proceptive behavior in female capuchin monkeys (*Cebus apella*). *Behav. Brain Res.,* **179**: 33-42.

Tripoli E, Guardia ML, Giammanco S, Di Majo D and Giammanco M (2007). Citrus flavonoids: Molecular structure, biological activity and nutritional properties: A review. *Food Chemistry,* **104**: 466-479.

Turk G, Sonmez M, Aydin M, Yuce A, Gur S, Yuksel M, Aksu EH and Aksoy H (2008). Effects of pomegranate juice consumption on sperm quality, spermatogenic cell density, antioxidant activity and testosterone level in male rats. *Clinical Nutrition,* **27**: 289-296.

Viuda-Martos M, Ruiz-Navajas Y, Fernandez-Lopez J and Perez-Alvarez J (2008). Antifungal activity of lemon (Citrus lemon L.), mandarin (*Citrus reticulata* L.), grapefruit (*Citrus paradisi* L.) and orange (*Citurs sinensis* L.) essential oils. *Food Control,* **19**: 1130-1138.

Wang CC, Prasain JK and Barnes S (2002). Review of the methods used in the determination of phytoestrogens. *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.,* **777**: 3-28.

Waheed S, Siddique N, Rahman A, Zaidi JH and Ahmed S (2004). INAA for dietary assessment of essential and other trace elements in 14 fruits harvested and consumed in Pakistan. *J. Radioloanal. Nucl. Chem.,* **260**: 523-531.

Wayne PA (1998). Collection, transport and processing of blood specimens for coagulation testing and general performance of coagulation assays. Approved guideline H21-A2, 3rd Edition, National Committee for Clinical Laboratory Standards, 1998. (NCCLS Document H21-A3).

Wi S, Gong Z, Ma W and Wei M (2012). Effects of GnRH agonist on reproductive functions of female rabbits. *Animal Science papers and Reports,* **30**: 73-86.

Wilcox J Lisa, Borradaile M Nica and Huff W Murray (1999). Antiatherogenic properties of naringenin, a citrus flavonoid. Cardiovascular drug reviews. **17**: 160-178.

Wilson ME and Handa RJ (1997). Ontogeny of pituitary gene expression in the infantile female rat. *Biology of Reproduction,* **56**: 563-568.

Wilson ME and Handa RJ (1998). Direct Actions of Gonadal Steroid Hormones on FSH Secretion and Expression in the Infantile Female Rat. *J. Steroid Biochem. Molec. Biol.,* **66**: 71-78.

Yu Y, Li W, Han Z, Luo M, Chang Z and Tan J (2003). The effect of follicle-stimulating hormone on follicular development, granulosa cell apoptosis and steroidogenesis and its mediation by insulin-like growth factor-I in the goat ovary. *Theriogenology,* **60**: 1691-1704.