ISSN NO. 2320-5407

*Journal homepage: http://www.journalijar.com*

*INTERNATIONAL JOURNAL OF ADVANCED RESEARCH*

**RESEARCH ARTICLE**

# EFFECT OF AGE AND SEX HORMONES ON SERUM LEVELS OF LIPOCALIN-2 IN FEMALE ALBINO RATS

**Ahmed B-E. Abd-Allah, Salah M. I. Zaghloul, Abeer Albiomy Khalifa, Wesam Mohamed Reda Mahmoud**
Department of physiology, Faculty of Medicine _ Zagazig University

## *Manuscript Info*

***Manuscript History:***

Received: 18 December 2015
Final Accepted: 22 January 2016
Published Online: February 2016

***Key words:***
Lipocalin 2, age, sex, estrogen, rats.

***Corresponding Author***
**Ahmed B-E. Abd-Allah.**

## *Abstract*

**Background:**-Lipocalin 2 has been implicated in diversified functions such as apoptosis, iron transport, inflammation, cell survival and innate immunity. Lipocalin 2 expression is affected by fat depot but the effect of age and sex hormones on the circulating lipocalin 2 levels in female rats is still unknown.

**Aim:-** To explore the effect of age and hypothalamo-pituitary-ovarian axis (HPOA) on Lipocalin 2 levels in albino rats.

**Material and Methods:-** The study was done on 90 female albino rats divided into 2 main equal groups: Group I, it was further subdivided into 3equal subgroups: Group I A:" pre-pubertal age". Group I B: "young Adult" (3 months old). Group I C:" Old age group" Group II (Ovariectomized "OVX"), it was further subdivided into 3equal subgroups: Group II A: Shame operated group, group II B: OVX group and group II C OVX with hormonal replacement group.

**Results:-** In intact group (group I), lipocalin 2 levels in young adult group were significantly higher than that of pre-pubertal and old age groups. And old age group showed non-significant change in relation to the pre-pubertal group.

In OVX group (group II) , lipocalin 2 levels in OVX group were significantly lower than that of young adult and shame operated groups and in the OVX group with hormonal replacement. Multiple regression analysis revealed that, estrogen is the major factor linked to the changes in lipocalin 2 levels.

**Conclusion:-** In female rats age and estrogen hormone are the apparent regulatory factors of the lipocalin 2 levels



## Introduction:-

Lcn 2 is a small protein of 25 kDa [1,2], was initially identified as a secreted protein from human neutrophils [3,4] and considered as a representative of a large group of lipocalins, which are small extracellular proteins with a variety of functions [5].

Lcn 2 is expressed in multiple tissues including kidney, brain, lung, liver, spleen, neutrophils, uterus, adipocytes and macrophages[4,6-8] and was involved in varieties of biological functions such as apoptosis, tumorigenesis and innate immunity, transport of fatty acid or iron [9-12]. Actively participates in the process of proliferation, development and

differentiation of different human tissues [13-15]. Also act as iron transporter as well as an antioxidant[16].suppression of bacterial growth[17], and modulator of inflammatory responses[18].

Adipose Lcn 2 expression is up-regulated in various models of mouse obesity and insulin resistance and in visceral compared with subcutaneous adipose tissue of obese humans [19] and its expression is gender and fat depot dependent [20]. In addition, previous studies reported that serum lipocalin-2 concentrations in males are significantly higher than those in females in both obese and lean subjects [21,22].

Lcn 2 deficiency leads to decreases in 17β- estradiol concentration in sera and estrogen receptor-α (ERα) signaling but the mechanisms remain unclear [20].

However, the effect of age and sex hormones on the circulating lipocalin 2 in female rats is not studied yet.

The aim of this research is to investigate the possible effect of age and sex hormones on serum Lcn 2 level in female albino rats.

## Material and methods:-

The rats (100 healthy female albino) were accommodated to laboratory conditions for three weeks before starting the experimental regimen [23]. Rats fed normal (standard) diet and had free access to water.

All rats received care in accordance with the national health guidelines and the study protocol was approved by the Institutional Review Board and ethics committee of faculty of medicine zagazig University.

The rats were divided into 2 main groups: Group I "Intact group" (Consists of 45 rats). t was further subdivided into 3equal subgroups: group IA:" pre-pubertal age" (4 weeks old- body weight, 60-80 gm), group IB: "young Adult" (3 months old- body weight, 180-200 gm) and group IC:" Old age group" (25 months old- body weight, 250-300 gm).

Group II" OVX group" (Consists of 55 rats "25 months old" body weight, 180-200 gm). Rats were further subdivided into 3 subgroups: group IIA: Shame operated group (n= 15) treated with 0.2 ml sesame oil (ADWIC Laboratory Chemicals, Egypt), group IIB: OVX group (treated with 0.2 ml sesame oil). (n= 20) {3 rats died "15%" 15 rats were selected from the remaining living 17rats according to IRB recommendation} and group IIC OVX with hormonal replacement group. (n= 20), rats were treated with estradiol (Sigma) in a dose of 10 µg/kg/day dissolved in 0.1 ml sesame oil and progesterone (sigma) in a dose of 10mg/kg/day dissolved in 0.1 ml sesame oil [24]. 1 rat died "5%" 15 rats were selected from the remaining living 19 rats according to IRB recommendation).

**Methods:-**

**Ovariectomy technique:-** [25].

Rats were fasted over night, they were anaesthetized by intraperitoneal (ip) with pentobarbital sodium (40 mg/kg body weight), and rats were tied to the operating board.

The hair removed from the lower abdomen. The bare area of the skin sterilized .Then a midline incision was done through the skin and the abdominal wall, after that the ovaries had been explored and removed surgically.

Abdominal wall was repaired with catgut thread, and then the skin incision had been closed with sterile silk suture. The closed incision painted with garamycin cream and covered with sterilized gauze, and then the rats were observed until the recovery from anesthesia.

**Sham operation technique:-**

The same procedure was done as ovarectomy technique without removal of the ovaries.
Replacement begun one week after surgical procedures and continue for 14 days [24,26].

**Determination of the estrous phases:-**

The mean duration of the estrous cycle was 4-5 days for 60%-70% of female rats and is characterized as: proestrus, estrus, metestrus and diestrus, which may be determined according to the cell types observed in the vaginal smear [27].

Vaginal secretion was collected with a plastic pipette filled with 10 mL of normal saline (NaCl 0.9%) by inserting the tip into the rat vagina, but not deeply. One drop was collected with a clean tip from each rat. Vaginal fluid was placed on glass slides. Unstained material was observed under a light microscope. The determination of the estrous cycle phases as follow:

The proestrus phase: predominance of nucleated epithelial cells with smooth margins.
The estrus phase: the presence of large anucleated cornified (keratinized) cells with irregular margins.
The met estrus phase: Many cornified cells plus infiltration of leukocytes.
The diestrus phase: absence of the cornified cells and presence of small leukocytes [27].

At the end experiment body mass index (BMI) was estimated by the equation: body weight (g) /lenght$^2$ (cm$^2$) = BMI (gm/cm$^2$) [28]. And then after overnight fasting , blood samples were obtained from sinus orbitus vein of each rat in the diestrus phase after ether inhalation. The blood samples were allowed to clot at room temperature before centrifuging at approximately 3000 rpm for 15 minutes. The serum was stored at -20° C.

Hormonal Assay was done by enzyme immunoassay test kits.
Serum Lipocalin-2: according to Goetz et al. [28]
Serum Gonadotropin releasing hormone (GnRH): according to Kataria et al. [30].
Serum Estradiol (E2), Progesterone and Luteinizing hormone (LH): according to Tietz [31].
Serum Follicle-Stimulating Hormone (FSH) according to Rebar et al. [32].
And all kits from BioCheck, Inc 323 Vintage Park Dr. Foster City, CA 94404)

**Statistical analysis:-**
The data obtained in the present study were expressed as mean ± SD for quantitative variables, one way ANOVA with LCD was done to compare means. Pearson correlation and multiple regression analysis were done (P value less than 0.05 was considered significant). The statistical analysis was done by using SPSS program (version 18 for windows) (SPSS Inc. Chicago, IL, USA).

## Results:-

Group IB (young adult group), while the mean values of serum levels of Lcn2, GnRH, estrogen, progesterone and calculated BMI were significantly high ($P < 0.001$), the mean values of serum levels of FSH and LH were significantly low ($P < 0.001$ and $P < 0.01$ respectively) in comparison to that of group IA (prepubertal group).

In group IC (old age group) the mean values of serum levels of Lcn2, FSH, LH and progesterone showed non-significant change when compared with those of group IA ($P > 0.05$). However, the mean values of serum levels of GnRH, estrogen and calculated BMI were significantly high ($P < 0.001$, $P < 0.05$ and $P < 0.001$ respectively). Moreover, in group IC the mean value of serum levels of Lcn2, estrogen and progesterone were significantly low ($P < 0.001$) when compared with those of group IB. But the mean values of serum levels of GnRH, FSH, LH and calculated BMI, were significantly high ($P < 0.001$).

There were no significant changes in all parameters measured between sham operated group (IIA), intact young adult group (IB) and OVX with hormonal replacement group (IIC) ($P > 0.05$).

In group IIB (OVX group) although the mean values of serum levels of Lcn2, estrogen and progesterone were significantly low ($P < 0.001$), the mean values of serum levels of GnRH, , FSH, LH and calculated BMI were significantly high ($P < 0.001$, $P < 0.001$, $P < 0.01$ and $P < 0.001$ respectively) in comparison to that of group IB.

In group IIC the mean values of serum levels of Lcn2, estrogen and progesterone were significantly high ($P < 0.001$). Conversely, the mean values of serum levels of GnRH, FSH, LH and calculated BMI were significantly low ($P < 0.001$) when compared with those of group IIB.

As regards the correlation with Lcn2 levels, estrogen and progesterone were significantly positive correlated with Lcn2 in all studied groups. Whereas, GnRh was significantly positive correlated with Lcn2 in all groups except IC and IIB groups, BMI was significantly positive correlated with Lcn2 in all groups except IC.

Furthermore, FSH and LH were significantly negative correlated with Lcn2 in all studied groups.

Multiple regression analysis revealed that, estrogen is a major factor linked to the change in lipocalin 2 levels as the significance was less than 0.001(0.000) in all groups , while the significance of other parameters (BMI, GnRH, FSH, LH and Progesterone) in the pre-pubertal were 0.048, 0.126, 0.958, 0.583 respectively, and 0.421 respectively, in young adult group were 0.204, 0.016, 0.013, 0.009 and0.004 respectively, in old age group were 0.168, 0.442, 0.508, 0.118 and 0.006 respectively, in OVX group were 0.512, 0.499, 0.436, 0.788 and 0.878 and in group OVX with replacement were 0.648, 0.963, 0.118, 0.379 and 0.434 respectively.

**Table 1**: serum lipocalin-2, GnRH, FSH, LH, estrogen, progesterone, and calculated BMI in all studied groups:-

| N= 15 | | IA (pre-pubertal) | IB (young Adult) | IC (Old age) | IIA (Shame operate) | IIB (OVX.) | IIC (OVX + replacement) |
|---|---|---|---|---|---|---|---|
| Lcn2 (ng/ ml) | X ±SD | 31.43 ±0.8 | $58.78^{a}$ ±1.3 | $32.76^{b}$±0.9 | 56.53 ±1.1 | $32.33^{bc}$ ±0.9 | $56.67^{d}$ ±1.00 |
| BMI ($gm/cm^2$) | X ±SD | 0.43 ±0.007 | $0.59^{a}$ ±0.007 | $0.69^{ab}$ ±0.007 | 0.59 ±0.008 | $0.67^{bc}$ ±0.008 | $0.57^{d}$ ±0.007 |
| | R | $0.663^{**}$ P < 0.01 | P < 0.05 | 0.299 NS | $0.692^{**}$ P < 0.01 | 0.668** P < 0.01 | $0.682^{**}$ P < 0.01 |
| GnRH (pg/ ml) | X ±SD | 39.84 ±0.7 | $76.78^{a}$ ±1.4 | $89.75^{ab}$ ±1.2 | 75.96 ±1.6 | $87.39^{bc}$ ±1.4 | $77.17^{d}$ ±1.6 |
| | R | $0.712^{**}$ P < 0.01 | $0.621^{*}$ P < 0.05 | 0.110 NS | $0.531^{*}$ P < 0.05 | 0.233 NS | $0.687^{**}$ P < 0.01 |
| FSH (□ IU/L) | X ±SD | 4.50 ±0.1 | $3.71^{a}$ ±0.08 | $4.55^{b}$ ±0.2 | 3.70 ±0.09 | $5.63^{bc}$ ±0.2 | $3.64^{d}$ ±0.07 |
| | R | $-0.544^{*}$ P < 0.05 | $-0.525^{*}$ P < 0.05 | $-0.518^{*}$ P < 0.05 | $-0.613^{*}$ P < 0.05 | $-0.661^{**}$ P < 0.01 | $-0.696^{**}$ P < 0.01 |
| LH (□ IU/L) | X ±SD | 3.40 ±0.1 | $2.64^{a}$ ±0.09 | $3.67^{b}$ ±0.2 | 2.69 ±0.08 | $3.69^{bc}$ ±0.2 | $2.37^{d}$ ±0.1 |
| | R | $-0.569^{*}$ P < 0.05 | $-0.631^{*}$ P < 0.05 | $-0.621^{*}$ P < 0.05 | $-0.537^{*}$ P < 0.05 | $-0.645^{**}$ P < 0.01 | $-0.559^{*}$ P < 0.05 |
| estrogen (pg/ ml) | X ±SD | 6.67 ±0.4 | $29.90^{a}$ ±1.3 | $9.24^{b}$ ±0.2 | 29.31 ±1.2 | $6.86^{bc}$ ±0.4 | $29.09^{d}$ ±1.04 |
| | R | $0.983^{***}$ P < 0.001 | $0.978^{***}$ P < 0.001 | $0.967^{***}$ P < 0.001 | $0.957^{***}$ P < 0.001 | $0.981^{***}$ P < 0.001 | $0.982^{***}$ P < 0.001 |
| Progesterone (ng/ ml) | X ±SD | 1.83 ±0.09 | $9.80^{a}$ ±0.5 | $2.62^{b}$ ±0.1 | 10.14 ±0.4 | $1.70^{bc}$ ±0.09 | $10.02^{d}$ ±0.4 |
| | R | $0.569^{*}$ P < 0.05 | $0.612^{*}$ P < 0.05 | $0.710^{*}$ P < 0.05 | $0.699^{**}$ P < 0.01 | $0.626^{*}$ P < 0.05 | $0.694^{**}$ P < 0.01 |

a = significant VS group IA b= significant VS group IB
c= significant VS group IIA d = Significant VS group IIB

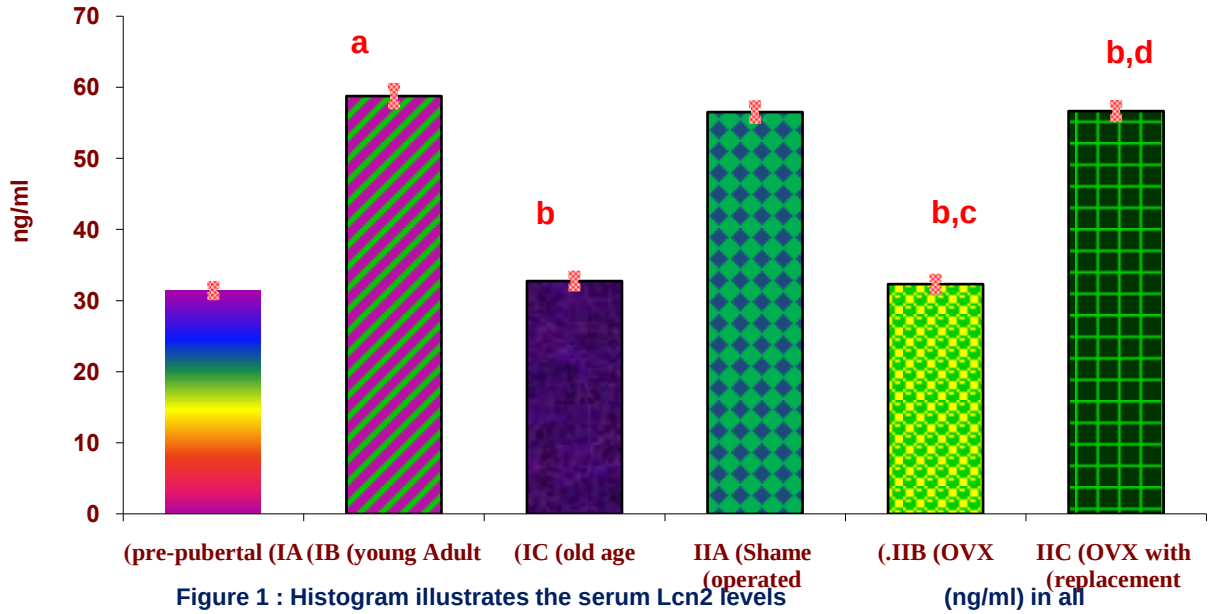


**Figure 1 : Histogram illustrates the serum Lcn2 levels (ng/ml) in all studied groups.**

**a = significant VS group IA b= significant VS group IB**
**c= significant VS group IIA d = Significant VS group IIIB.**

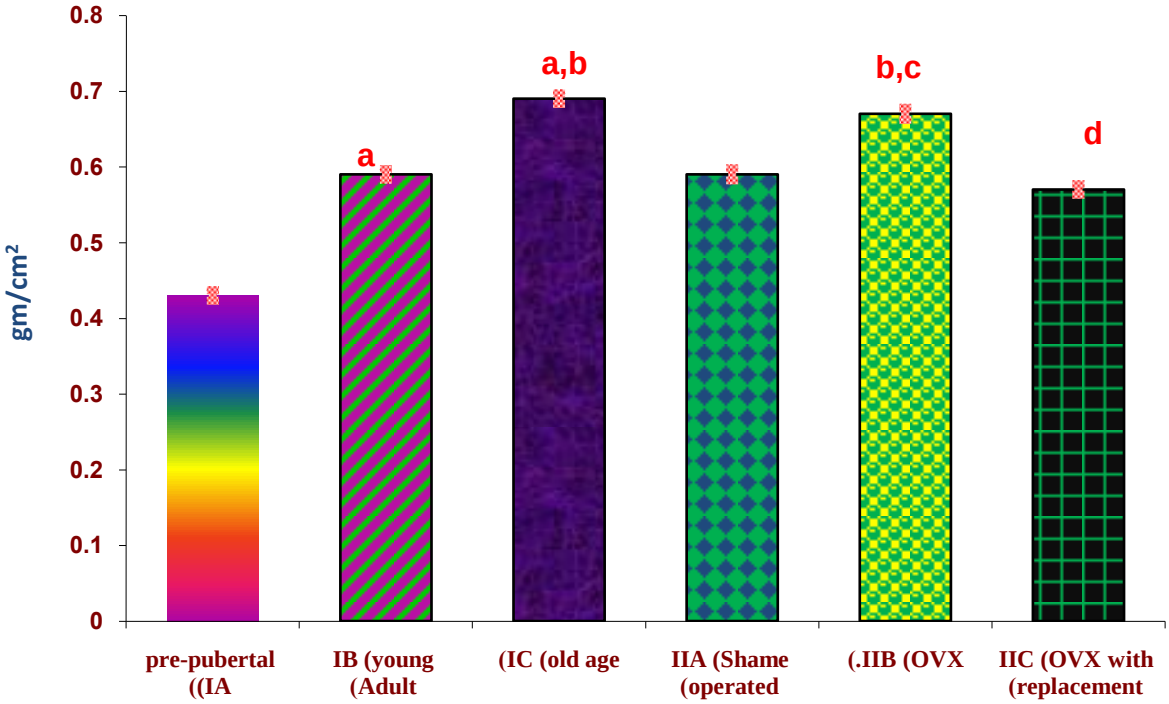


**Figure 2: Histogram illustrates calculated BMI (gm/cm²) in all tudied groups.**

**a = significant VS group IA b= significant VS group IB**
**c= significant VS group IIA d = Significant VS group IIB.**

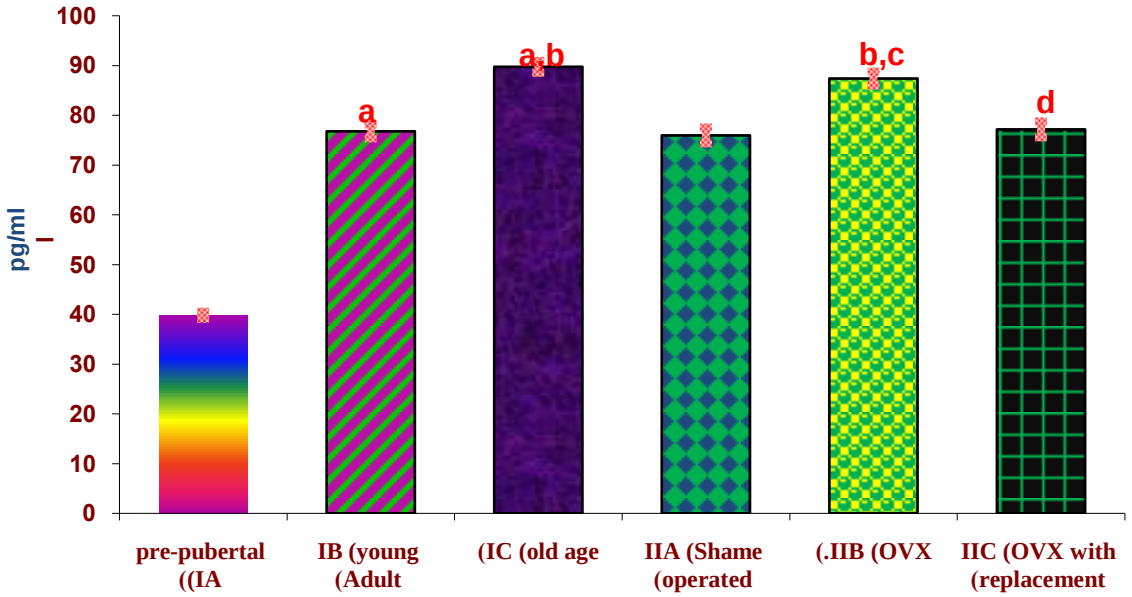


**Figure 3 : Histogram illustrates the serum GnRH levels (pg/ml) in all studied groups.**

**a = significant VS group IA b= significant VS group IB**
**c= significant VS group IIA d = Significant VS group IIB.**

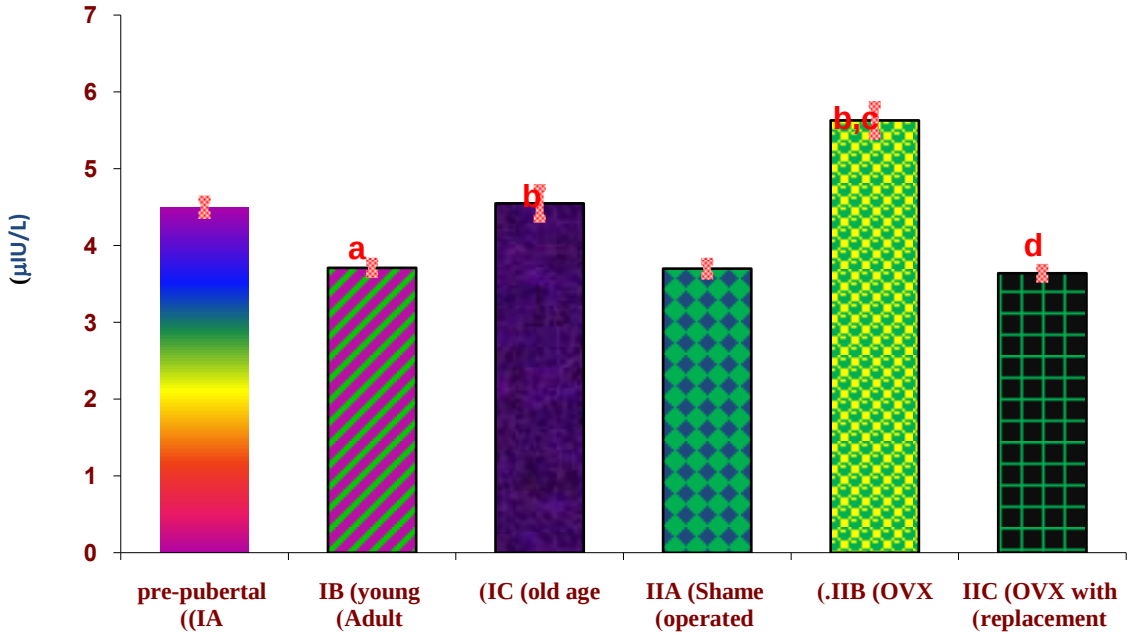


**Figure 4 : Histogram illustrates the serum FSH levels (µIU/L in all studied groups .**

**a = significant VS group IA b= significant VS group IB**
**c= significant VS group IIA d = Significant VS group IIB.**

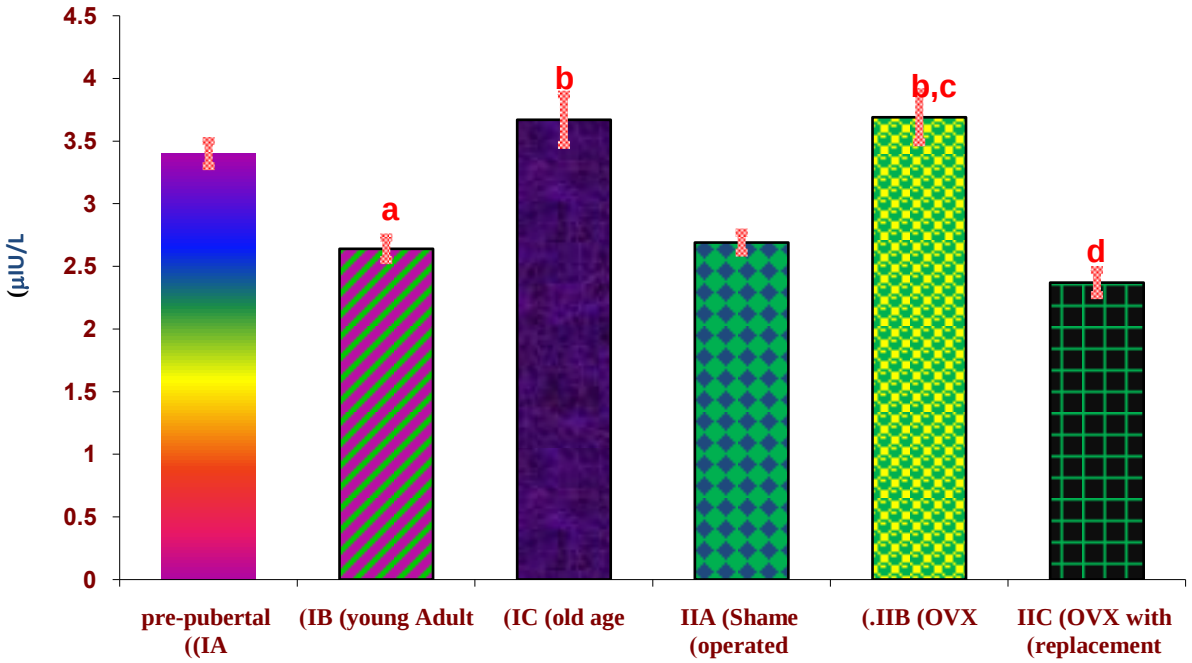


**Figure 5 : Histogram illustrates the serum LH levels (µIU/L )in all studied groups .**

**a = significant VS group IA b= significant VS group IB**
**c= significant VS group IIA d = Significant VS group IIB.**

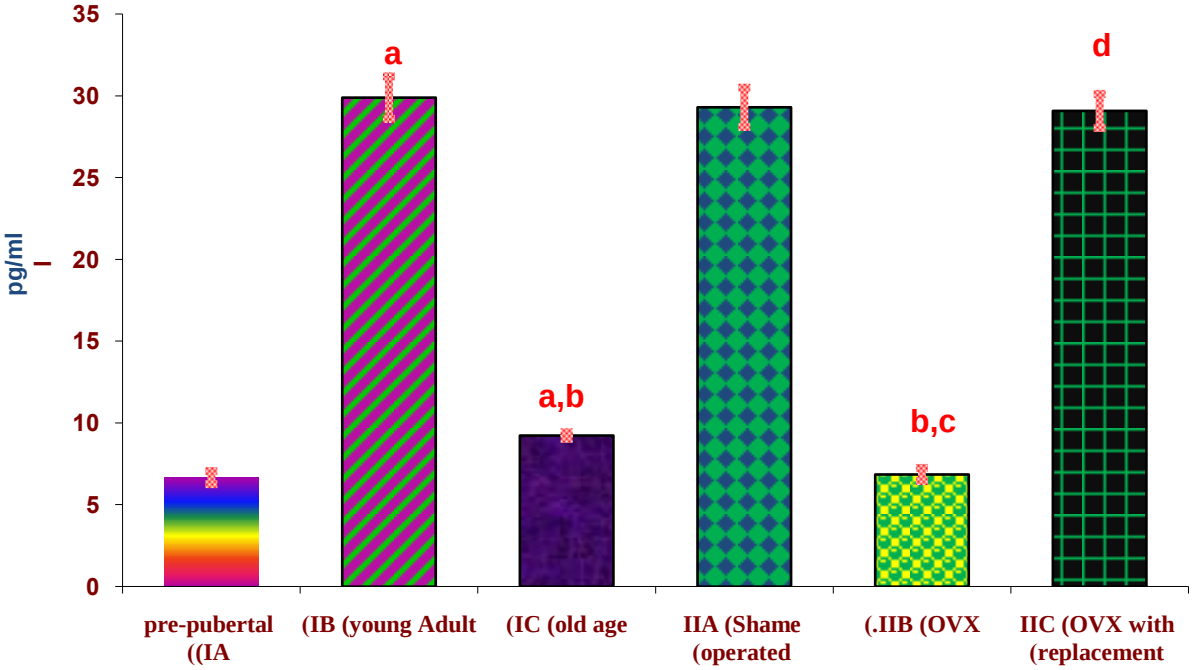


**Figure 6: Histogram illustrates the serum estrogen levels ( pg/ml) in all studied groups .**

**a = significant VS group IA b= significant VS group IB**
**c= significant VS group IIA d = Significant VS group IIB.**

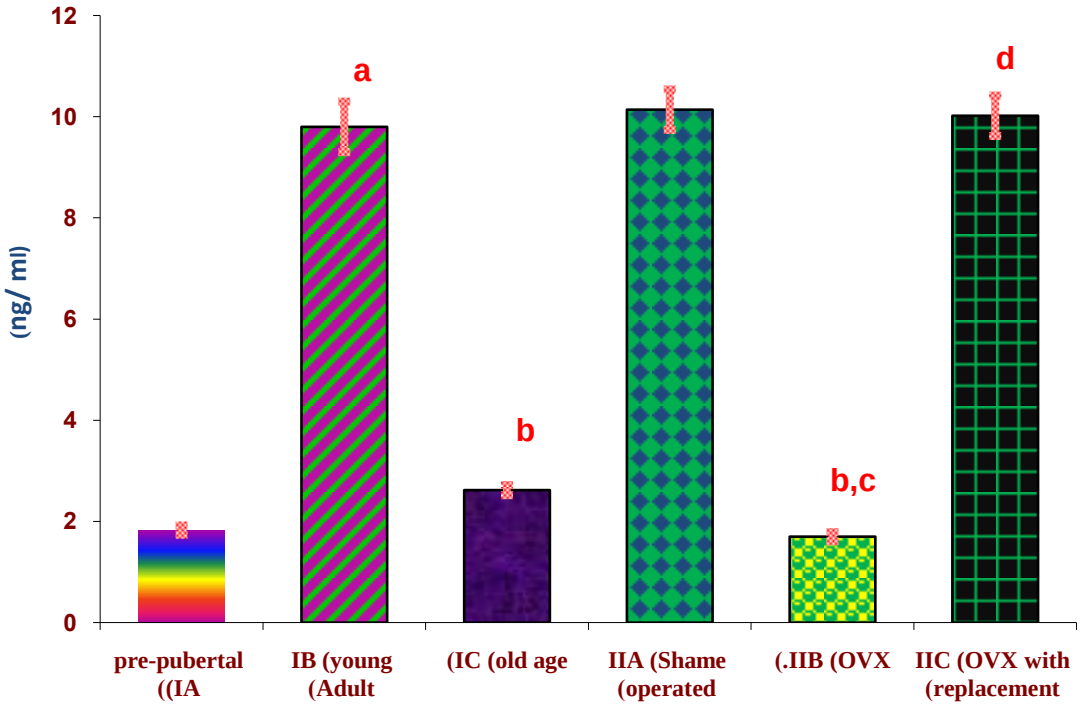


**Figure 7 : Histogram illustrates the serum progesterone levels (ng/ml) in all studied groups .**
**a = significant VS group IA b= significant VS group IB**
**c= significant VS group IIA d = Significant VS group IIB.**

## Discussion:-

Lcn 2 plays a significant role in obesity, insulin resistance, brown adipose tissue activation [33]. This study was designed to examine the effect of age and HPOA on the circulating lipocalin 2 levels in female rats.

In the present study, as regards the effect of age (group I), serum Lcn 2 levels in young adult group were significantly higher than that of prepubertal and old age groups.

The increased serum Lcn 2 levels in young adult group more than the prepubertal group can be explained by different variables as the difference in calculated BMI which proved in this study as calculated BMI correlated positively with serum Lcn 2 levels in prepubertal and young adult groups and these results are in agreement with [21, 34,35] who found a high concentration of Lcn 2 in obesity and positive correlation between serum Lcn 2 concentrations and BMI both in humans and animals. Moreover, Rendárová et al. [36] found a positive correlation between serum Lcn 2 and BMI in patients with psoriasis.

However, others have found similar circulating Lcn 2 levels in obese and lean subjects [37,38]. Moreover, studies in healthy young men by Liu et al. [39] and two groups of obese children by Kanaka-Gantenbein et al. [40]. Akelma et al. [41] also concluded that plasma Lcn 2 was not related to metabolic parameters. Obese and non-obese diabetic individuals showed reduction of Lcn 2 levels in comparison to healthy subjects [42,43]

Unfortunately, the BMI alone cannot explain the differences in circulating Lcn 2 levels with age as in old age BMI increased but lipocalin 2 levels are significantly decreased.

So the possible mechanism of this change in Lcn 2 levels with age can be sex hormone mediated, therefor, the relation between serum Lcn 2 levels and HPOA hormones was investigated in this research.

In the present research Serum GnRH levels in young adult group were significantly higher than that of prepubertal group. Moreover, in old age group they were significantly higher than that of prepubertal and young adult groups.

There was significant positive correlation between GnRH and Lcn 2 levels in prepubertal and young adult groups. These results are supported by the fact that GnRH secretion is increased at the time of puberty onset leading to high frequency pulses that induce the awakening of the reproductive function [(44)]. In addition, Gore et al. [(45)] found that, GnRH mRNA levels increase significantly with aging, with higher levels in middle-aged and old than in young rats. Furthermore, Gill et al. [(46)] and Shaw et al. [(47)] reported that, the pituitary response to GnRH was decreased with aging in women. GnRH increases with aging, although GnRH pulse frequency decreases. In addition, Gore et al. [(45)] found that, in old persistent diestrus rats, GnRH gene expression was increased. GnRH mRNA levels were higher in postmenopausal women than premenopausal women [(48)].

Additionally, older menopausal women exhibit decreased GnRH pulse frequencies but an increase in overall GnRH secretion when compared to younger menopausal women [(49)].

In contrast, GnRH mRNA levels decreased in old and young Sprague-Dawley rats in diestrus. GnRH peptide concentrations decreased in the median eminence of middle-aged compared with young rats at the beginning of the LH surge on proestrus [(50)]

Moreover, age-related changes to the hypothalamic GnRH neurons in rodents, include diminished GnRH release, neural activation, and an attenuated preovulatory GnRH/LH surge [(51)]

Estrogen showed significant positive correlation with GnRH in old age, young adult and OVX groups. The relation between estrogen and GnRH was explored previously by a positive as well as a negative effect on GnRH secretion. Estrogen suppresses GnRH secretion in a negative-feedback fashion but prior to ovulation estrogen's influence switch to a positive feedback mechanism leading to the GnRH/LH surge[(52,53)].

As regards the FSH and LH hormones, serum FSH and LH levels in young adult group were significantly lower than that of prepubertal and old age groups Moreover, in this research, there was significant negative correlation between FSH and LH and Lcn 2 levels in the three subgroups. This is in line with elevated serum gonadotropin levels during the juvenile period despite the absence of pulsatile GnRH secretion[(54)].

LH and FSH secretion are both regulated by GnRH release from the hypothalamus. Moreover, FSH is also regulated by ovarian inhibins and activins. The increase in FSH levels seen as an early marker of menopausal progression is likely due to a decrease in circulating inhibins and increases in activins and may be better associated with age than with menopausal status[(53,55)]. However, Matt et al [(56)].stated that serum LH levels decreased with chronological age on proestrus and underwent more gradual decreases as middle-aged rats transitioned into acyclicity.

As regards serum estrogen and progesterone levels, they were significantly higher in young adult group than prepubertal and old age groups. In old age group the serum estrogen levels were significantly higher than that of prepubertal group. Ovarian decline occurs between six and eighteen months and is characterized by low levels of estradiol and progesterone, with little or no developing follicles[(57)], and the decreased steroid secretion by the ovaries may be causally related to the lack of preovulatory increases in gonadotropin and prolactin release in aging rats[(58,59)]. Serum estradiol concentrations at the pubertal and post-pubertal ages were significantly higher than those at the prepubertal age[(60)].Also progesterone levels declined with aging, and urinary progesterone metabolites were decreased in perimenopausal women[(53,61)]. However, Gore et al. [(45)] reported that, aging rats do not experience follicular atresia, and estradiol levels do not decrease with aging in the rat and found overall increase in circulating estradiol levels with aging.

Moreover, there was significant positive correlation between lipocalin 2 levels and both of estrogen and progesterone in the three subgroups. This is supported by Kim et al. [(62)] who stated a positive correlation between estrogen and Lcn 2 production and/or action in controlling adipose lipid metabolism.

As regard effect of ovarectomy, serum Lcn 2 levels in OVX group were significantly lower than that of young adult group. Moreover, serum Lcn 2 levels in shame operated group were significantly higher than that of OVX group. In addition, serum Lcn 2 levels in OVX group with hormonal replacement were significantly higher than that of OVX group.

However, there was no significant change in serum Lcn 2 levels in shame operated group in comparison to OVX group with hormonal replacement and these two groups show insignificant change in serum Lcn 2 levels when compared with young adult group.

As regards calculated BMI, calculated BMI in OVX group was significantly higher than that of young adult and OVX groups with hormonal replacement. However, there was no significant change in calculated BMI in shame operated group and OVX group with hormonal replacement in comparison with young adult group. This is in agreement with Ross and Howlett [63] who revealed that, OVX female rats show higher BMI than control.

In addition, in the present study, there was significant positive correlation between calculated BMI and Lcn 2 levels in shame operated, OVX and OVX with hormonal replacement groups.

Serum GnRH levels in OVX group were significantly higher than that of young adult and shame operated groups and OVX group with hormonal replacement. However, there was no significant change in serum GnRH levels in shame operated group and OVX group with hormonal replacement when compared with young adult group.

The absence of negative feedback of sex steroids after gonadectomy and menopause [64] associated with a 2-3-fold rise in GnRH receptors coincident with the rise in serum gonadotropins and these responses can be prevented by immunoneutralization with a specific GnRH antiserum suggesting that increased endogenous GnRH is essential for the post-gonadectomy rise in pituitary GnRH receptors and serum gonadotrophins [65].

Estrogen and progesterone treatment of OVX mice suppressed both high serum LH and FSH levels while increasing GnRH receptors[66].

However, in old acyclic rats, the pulsatile release of GnRH is blunted after prolonged periods of estradiol withdrawal[44, 67].

As regards FSH and LH hormones, serum FSH and LH levels in OVX group were significantly higher than that of young adult and shame operated group and OVX group with hormonal replacement. However, there was no significant change in serum FSH and LH levels in shame operated group and OVX group with hormonal replacement when compared with young adult group. Moreover, FSH and LH levels in shame operated group showed non-significant change in comparison to OVX group with hormonal replacement.

The concentrations of LH and FSH in the circulation gradually rise to reach a ten-fold value and a plateau 2–3 weeks after the OVX. And if OVX animals are treated with exogenous progesterone, LH and FSH concentrations decrease rapidly to the tonic levels[68]. Moreover on the day after hemiovariectomy, serum FSH levels elevated in both old and young rats and after a suppressive dose of estradiol benzoate, OVX old rats showed lower serum LH levels than did young OVX rats[69].

In this research, there was significant negative correlation between FSH and LH and Lcn 2 levels in shame operated, OVX and OVX with hormonal replacement groups.

As regards, estrogen and progesterone hormones, serum levels in OVX group were significantly lower than that of young adult and shame operated group and OVX group with hormonal replacement.

In addition, there was significant positive correlation between estrogen and Lcn 2 levels in shame operated, OVX and OVX with hormonal replacement groups in our work.

These increase in Lcn 2 levels with estrogen as 17β-estradiol increases circulating levels of Lcn 2 and its expression in white adipose tissue [70]. Moreover, and treatment with therapeutic doses of 17β-estradiol increases the serum lipocalin 2 levels in OVX mice[62]. Lcn 2 was induced in the endometrial epithelial cells by estrogen[71]. Moreover, Lcn 2 deficiency may cause dysregulation of systemic and adipose lipid metabolism by affecting the production and action of estrogens [72] reported that. Furthermore, statistically significant stimulatory effects of Lcn 2 and resistin on the synthesis of ovarian steroids was detected [73].

Multiple regression analysis based on the regulation of Lcn 2 levels in al studied groups (group I and group II) revealed highest significance with estrogen hormone.

The regulatory effect of estrogen on Lcn 2 can be considered as one of the multiple anti-inflammatory and anti-oxidant effects of estrogen by which it protects females from ischemic damage [(74)].

lipocalin family protein was highly expressed in the urine of a female rodent during estrus phase[(75)]. Moreover, Lcn 2 protect cells under oxidative stresses[(76)]. In addition, Lcn2 induces the expression of some growth factors, which are not only essential for survival and proliferation of mesenchymal stem cells but also augments them against harmful microenvironments [(77)].

Lcn 2 act as an antagonist to the effect of inflammatory molecules on secretion of adipokines. As the Lcn 2 up regulates PPARγ, increases the release of adiponectin and also antagonizes TNF-α effects on inflammatory and metabolic gene expression in adipocytes and macrophages[(78,79)]. Lcn 2 seems to protect against TNFα-induced insulin resistance in adipocytes. And increased production of Lcn 2 in obesity may be a protective mechanism against inflammation and insulin resistance[(33)]

In conclusion: In female rats, the circulating level of lipocalin 2 is age dependent. Estrogen hormone is the apparent modulating factors of the circulating lipocalin 2 levels

## References:-


1. Triebel S, Blaser J, Reinke H, Tschesche H: A 25 kDa a2-microglobulin-related protein is a component of the 125 kDa form of human gelatinase. FEBS Letters. 1992; 314, 386–388.
2. Guiddir T, Deghmane AE, Giorgini D, Taha MK: Lipocalin 2 in cerebrospinal fluid as a marker of acute bacterial meningitis. BMC Infect Dis. 2014; 20; 14:276.
3. Kjeldsen L, Johnsen AH, Sengelov H, Borregaard N: Isolation and primary structure of NGAL, a novel protein associated with human neutrophil gelatinase. J Biol Chem. 1993; 268, 10425–10432.
4. Zhang Y, Foncea R, Deis JA, Guo H, Bernlohr DA, Chen X: Lipocalin 2 expression and secretion is highly regulated by metabolic stress, cytokines, and nutrients in adipocytes. PLo S One. 2014; 12; 9(5): e96997. doi: 10.1371.
5. Helanova K, Spinar J, Parenica J: Diagnostic and prognostic utility of neutrophil gelatinase-associated lipocalin (NGAL) in patients with cardiovascular diseases--review. Kidney Blood Press Res. 2014; 39(6):623-629.
6. Aigner F, Maier HT, Schwelberger HG, Wallnöfer EA, Amberger A, Obrist P, Berger T, Mak TW, Maglione M, Margreiter R, Schneeberger S, Troppmair J: Lipocalin-2 regulates the inflammatory response during ischemia and reperfusion of the transplanted heart. Am. J. Transplant. 2007; 779–788.
7. Flo TH, Smith KD, Sato S, Rodriguez DJ, Holmes MA, Strong RK, Akira S, Aderem A: Lipocalin 2 mediates an innate immune response to bacterial infection by sequestrating iron. Nature. 2004; 432(7019): 917-921.
8. Huang Y, Yang Z, Ye Z, Li Q, Wen J, Tao X, Chen L, He M, Wang X, Lu B, Zhang Z, Zhang W, Qu S, Hu R: Lipocalin-2, glucose metabolism and chronic low-grade systemic inflammation in Chinese people. Cardiovasc Diabetol. 2012; 31; 11:11. doi: 10.1186/1475-2840-11-11.
9. Yang J, Bielenberg DR, Rodig SJ, Doiron R, Clifton MC, Kung AL, Strong RK, Zurakowski D, Moses MA: Lipocalin 2 promotes breast cancer progression. Proc. Natl. Acad. Sci. U. S. A. 2009; 106, 3913-3918.
10. Berger T, Cheung CC, Elia AJ, Mak TW: Disruption of the Lcn2 gene in mice suppresses primary mammary tumor formation but does not decrease lung metastasis. Proc. Natl. Acad. Sci. U. S. A. 2010; 107, 2995-3000.
11. Zhang Y: Understanding the role of Lipocalin 2 as an important regulator of energy metabolism. University of Minnesota Digital Conservancy. 2013. http://purl.umn.edu/148934.
12. Abella V, Scotece M, Conde J, Gómez R, Lois A, Pino J, Gómez-Reino JJ, Lago F, Mobasheri A, Gualillo O: The potential of lipocalin-2/NGAL as biomarker for inflammatory and metabolic diseases.. Biomarkers. 2015; 15:1-7. [Epub ahead of print]
13. Wenners AS, Mehta K, Loibl S, Park H, Mueller B, Arnold N, Hamann S, Weimer J, Ataseven B, Darb-Esfahani S, Schem C, Mundhenke C, Khandan F, Thomssen C, Jonat W, Holzhausen HJ, von Minckwitz G, Denkert C, Bauer M: Neutrophil gelatinase-associated lipocalin (NGAL) predicts response to

neoadjuvant chemotherapy and clinical outcome in primary human breast cancer. PLoS One. 2012; 7(10). doi: 10.1371/journal.pone.0045826.
14. Cabedo Martinez AI, Weinhaupl K, Lee WK, Wolff NA, Storch B, Żerko S, Konrat R, Koźmiński W, Breuker K, Thévenod F, Coudevylle N: Biochemical and structural characterization of the interaction between the Siderocalin NGAL/LCN2 and the N-terminal domain of its endocytic receptor SLC22A17.J Biol Chem. 2015;3. pii: jbc.M115.685644. [Epub ahead of print].
15. Ferreira AC, Dá Mesquita S, Sousa JC, Correia-Neves M, Sousa N, Palha JA, Marques F: From the periphery to the brain: Lipocalin-2, a friend or foe? Prog Neurobiol. 2015; (131):120-136.
16. Yamada Y, Miyamoto T, Kashima H, Kobara H, Asaka R, Ando H, Higuchi S, Ida K, Shiozawa T: Lipocalin 2 attenuates iron-related oxidative stress and prolongs the survival of ovarian clear cell carcinoma cells by up-regulating the CD44 variant. Free Radic Res. 2016; 4:1-36.
17. Goetz DH, Holmes MA, Borregaard N, Bluhm ME, Raymond KN, Strong RK: The neutrophil lipocalin NGAL is a bacteriostatic agent that interferes with siderophore-mediated iron acquisition, Mol Cell, 2002, 10(5), 1033-1043.
18. Kowsalya R, Jagatheesh K, Pavan Kumar Padarthi, Elangovan N: A functional approach of neutrophil gelatinase associated lipocalin (NGAL) – lipocalin family, International Journal of Bioassays. 2013; 02 (02), 483-487.
19. Fried SK, Greenberg AS: Lipocalin 2: a "sexy" adipokine that regulates 17beta-estradiol and obesity. Endocrinology. 2012; 153(4): 1582-1584.
20. Guo H, Zhang Y, Brockman D, HahnWBD,ChenX: Lipocalin 2 deficiency alters estradiol production and estrogen receptor signaling in female mice. Endocrinology. 2012; 153, 1183–1193.
21. Wang Y, Lam KS, Kraegen EW, Sweeney G, Zhang J, Tso AW, Chow WS, Wat NM, Xu JY, Hoo RL, Xu A: Lipocalin-2 is an inflammatory marker closely associated with obesity, insulin resistance, and hyperglycemia in humans. Clin Chem. 2007; 53, 34–41.
22. Luque-Ramírez M, Martínez-García MÁ, Montes-Nieto R, Fernández-Durán E, Insenser M, Alpañés M, Escobar-Morreale HF: Sexual dimorphism in adipose tissue function as evidenced by circulating adipokine concentrations in the fasting state and after an oral glucose challenge. Hum. Reprod. 2013; 28(7):1908-1918.
23. Gui Y, Silha JV, Murphy LJ: Sexual dimorphism and regulation of resistin, adiponectin, and leptin expression in the mouse. Obes. Res. 2004; 12,1481–1491.
24. Patra PB, Thorneloe KS, Laping NJ: Effect of estrogen and progesterone on urodynamics in the conscious rat. Urology. 2009; 74(2):463-466.
25. Irahara M, Tamura T, Matuzaki T, Saito S, Yasui T, Yamano S, Kamada M, Aono T: Orexin-A suppresses the pulsatile secretion of luteinizing hormone via β-endorphin. Biochemical and Biophysical Research Communications.2001; 281, 232-236.
26. Fan J, Yu LH, Zhang Y, Ni X, Ma B, Burnstock G: Estrogen altered visceromotor reflex and P2X (3) mRNA expression in a rat model of colitis.. Steroids. 2009; 74(12):956-962.
27. Marcondes FK, Bianchi FJ, Tanno AP: determination of the estrous cycle phases of rats: some helpful considerations. Braz. J. Biol. 2002; 62(4A):609-614.
28. Novelli EL, Diniz YS, Galhardi CM, Ebaid GM, Rodrigues HG, Mani F, Fernandes AA, Cicogna AC, Novelli Filho JL: Anthropometrical parameters and markers of obesity in rats. Laboratory Animals. 2007; 41, 111–119.
29. Goetz DH, Holmes MA, Borregaard N, Bluhm ME, Raymond KN, Strong RK: The neutrophil lipocalin NGAL is a bacteriostatic agent that interferes with siderophore-mediated iron acquisition. Molec. Cell. 2002; 10: 1033-1043.
30. Kataria H, Gupta M, Lakhman S, Kaur G: Withania somnifera aqueous extract facilitates the expression and release of GnRH: In vitro and in vivo study. Neurochem Int. 2015; 89, 111-119.
31. Tietz NW: Clinical Guide to Laboratory Tests, 3rd Ed., W.B. Saunders Company.; Philadelphia, 1995; PA 19106.
32. Rebar RW, Morandini IC, Petze JE, Erickson GF: Hormonal basis of reproductive defects in athymic mice: reduced gonadotropins and testosterone in males. Biol. Repro. 1982; 5, 1267-76.
33. Guo H, Jin D, Zhang Y, Wright W, Bazuine M, Brockman DA, Bernlohr DA, Chen X: Lipocalin-2 deficiency impairs thermogenesis and potentiates diet-induced insulin resistance in mice. Diabetes. 2010; 59, 1376–1385.
34. Damirchi A, Rahmaninia F, Mehrabani J: Lipocalin2: response to a short treadmill protocol in obese and normal weight men. Journal of human sport & Exercise. 2011; 6 (1): 59-66.

35. Mohammadi A, Alikhajehlandi, Reddy PV: Impact of aerobic exercise training on insulin resistance and plasma lipocalin 2 levels in obese young men. Biomedical & Pharmacology Journal. 2014; 7 (1):47-52.
36. Rendárová S, Ettler K, Borská L, Krejsek J, Andrýs C, Kremláček J, Řeháček V: Significance of serum levels of CRP, LP-PLA2, leptin and lipocalin 2 in patients With psoriasis. Čes-slov Derm. 2015; 4:157-163.
37. Catalán V, Gómez-Ambrosi J, Rodríguez A, Ramírez B, Silva C, Rotellar F, Gil MJ, Cienfuegos JA, Salvador J, Frühbeck G: Increased adipose tissue expression of lipocalin-2 in obesity is related to inflammation and matrix metalloproteinase-2 and metalloproteinase-9 activities in humans. J Mol Med. 2009; 87, 803–813.
38. Auguet T, Quintero Y, Terra X, Martínez S, Lucas A, Pellitero S, Aguilar C, Hernández M, del Castillo D, Richart C: Upregulation of lipocalin 2 in adipose tissues of severely obese women: positive relationship with proinflammatory cytokines. Obesity (Silver Spring). 2011; 19 (12): 2295-2300.
39. Liu X, Hamnvik OP, Petrou M, Gong H, Chamberland JP, Christophi CA, Kales SN, Christiani DC, Mantzoros CS: Circulating lipocalin 2 is associated with body fat distribution at baseline but is not an independent predictor of insulin resistance: the prospective Cyprus Metabolism Study. Eur. J. Endocrinol. 2011; 165, 805-812.
40. Kanaka-Gantenbein C, Margeli A, Pervanidou P, Sakka S, Mastorakos G, Chrousos GP, Papassotiriou I: Retinol-binding protein 4 and lipocalin-2 in childhood and adolescent obesity: when children are not just "small adults". Clin. Chem. 2008; 54, 1176-1182.
41. Akelma AZ, Abaci A, Ozdemir O, Celik A, Avci Z, Razi CH, Hizli S, Akin O: The association of serum lipocalin-2 levels with metabolic and clinical parameters in obese children: a pilot study. J. Pediatr. Endocrinol. Metab. 2012; 25, 525-528.
42. Alkharfy KM, Al-Daghri NM, Vanhoutte PM, Krishnaswamy S, Xu A: Serum retinol-binding protein 4 as a marker for cardiovascular disease in women. PLoS One. 2012; 7(10):e48612. doi: 10.1371/journal pone. 0048612.
43. De la Chesnaye E, Manuel-Apolinar L, Zarate A, Damasio L, Espino N, Revilla-Monsalve MC, Islas-Andrade S: Lipocalin-2 plasmatic levels are reduced in patients with long-term type 2 diabetes mellitus. Int J Clin Exp Med. 2015; 15; 8(2): 2853.
44. Navarro VM: New insights into the control of pulsatile GnRH release: the role of Kiss1/neurokinin B neurons .Front Endocrinol (Lausanne). 2012; 2, 3:48.
45. Gore AC, Oung T, Yung S, Flagg RA, Woller MJ: Neuroendocrine mechanisms for reproductive senescence in the female rat: gonadotropin-releasing hormone neurons.. Endocrine. 2000; 13(3):315-323.
46. Gill S, Sharpless J, Rado K, Hall J: Evidence that GnRH decreases with gonadal steroid feedback but increases with age in postmenopausal women. J Clin Endocrinol Metab. 2002; 87:2290–2296.
47. Shaw ND, Srouji SS, Histed SN, McCurnin KE, Hall JE: Aging attenuates the pituitary response to gonadotropin-releasing hormone. J Clin Endocrinol Metab. 2009; 94, 3259–3264.
48. Rance NE, Uswandi SV: Gonadotropin-releasing hormone gene expression is increased in the medial basal hypothalamus of postmenopausal women. J Clin Endocrinol Metab. 1996; 81, 3540– 3546.
49. Neal-Perry G, Nejat E, Dicken C. Maturitas: The neuroendocrine physiology of female reproductive aging: An update. 2010; 67(1) :34-38.
50. Li S, Givalois L, Pelletier G: Dehydroepiandrosterone administration reverses the inhibitory influence of aging on gonadotrophin-releasing hormone gene expression in the male and female rat brain. Endocrine. 1997; 6, 265–270.
51. Kermath BA, Riha PD, Woller MJ, Wolfe A, Gore AC: Hypothalamic molecular changes underlying natural reproductive senescence in the female rat. Endocrinology. 2014; 155(9): 3597-3609.
52. Beshay VE, Carr BR: Hypothalamic-Pituitary-Ovarian Axis and Control of the Menstrual Cycle. Clinical Reproductive Medicine and Surgery. 2013; 31-42, 10.1007/978-1-4614-6837-0_2.
53. Kermath BA, Gore AC: Neuroendocrine control of the transition to reproductive senescence: lessons learned from the female rodent model.. Neuroendocrinology. 2012; 96 (1):1-12.
54. Tezuka M, Irahara M, Ogura K, Kiyokawa M, Tamura T, Matsuzaki T, Yasui T, Aono T: Effects of leptin on gonadotropin secretion in juvenile female rat pituitary cells. Eur J Endocrinol. 2002; 146 (2): 261-266.
55. Hall JE: Neuroendocrine changes with reproductive aging in women. Seminars in Reproductive Medicine. 2007; 25 344–351.
56. Matt DW, Gilson MP, Sales TE, Krieg RJ, Kerbeshian MC, Veldhuis JD, Evans WS: Characterization of attenuated proestrous luteinizing hormone surges in middle aged rats by deconvolution analysis. Biology of Reproduction. 1998; 59 1477–1482.

57. Simpkins JW, Huang HH, Advis JP, Meites J: Changes in hypothalamic NE and DA turnover resulting from steroid- induced LH and prolactin surges in ovariectomized rats. Biology of Reproduction. 1979; 20, 625–632.
58. Rice KM, Fannin JC, Gillette C, Blough ER: Efficacy of female rat models in translational cardiovascular aging research.. J Aging Res. 2014; 2014:153127.
59. Lalithamma A, Changamma C: The effect of estradiol valerate on sex hormone levels in aged female albino rats. Int J Curr Pharm Res. 2015; 7, 36-38.
60. Yildirim M, Mapp OM, Janssen WG, Yin W, Morrison JH, Gore AC: Postpubertal decrease in hippocampal dendritic spines of female rats. Exp Neurol. 2008; 210 (2):339-348.
61. Santoro N, Brown JR, Adel T & Skurnick JH: Characterization of reproductive hormonal dynamics in the perimenopause. The Journal of Clinical Endocrinology and Metabolism. 1996; 81 1495–1501.
62. Kim JH, Cho HT, Kim YJ: The role of estrogen in adipose tissue metabolism: insights into glucose homeostasis regulation. Endocr J. 2014; 28; 61(11):1055-1067.
63. Ross JL, Howlett SE: Age and ovariectomy abolish beneficial effects of female sex on rat ventricular myocytes exposed to simulated ischemia and reperfusion. PLoS One. 2012; 7(6): 38425.
64. Gore AC,Windsor-Engnell B M, Terasawa E: Menopausal increases in pulsatile gonadotropin-releasinghormone release in a nonhuman primate (Macaca mulatta). Endocrinology . 2004; 145, 4653–4659.
65. Clayton RN, Popkin RM, Fraser HM: Hypothalamic regulation of pituitary gonadotrophin releasing hormone receptors. Effects of gonadotrophin releasing hormone immunoneutralization. Endocrinology. 1982; 110, 1116-1123.
66. Naik SI, Young LS, Saade G, Kujore A, Charlton HM, Clayton RN: Role of GnRH in the regulation of pituitary GnRH receptors in female mice. J Reprod Fertil. 1985; 74(2): 605-614.
67. Hall JE, Lavoie HB, Marsh EE, Martin KA: Decrease in gonadotropin-releasing hormone (GnRH) pulse frequency with aging in postmenopausal women. J Clin Endocrinol Metab. 2000; 85, 1794– 1800.
68. Pajović SB, Saicić ZS: Effects of ovarian hormones on the antioxidant enzymes in rat brain. Period boil. 2008; 110, 1.
69. Howland BE, Preiss C: Effects of aging on basal levels of serum gonadotropins, ovarian compensatory hypertrophy, and hypersecretion of gonadotropins after ovariectomy in female rats. Fertil Steril. 1975; 26(3): 271-276.
70. Kim JH, Meyers MS, Khuder SS, Abdallah SL, Muturi HT, Russo L, Tate CR, Hevener AL, Najjar SM, Leloup C, Mauvais-Jarvis F: Tissue-selective estrogen complexes with bazedoxifene prevent metabolic dysfunction in female mice. Mol Metab. 2014; 9; 3(2):177-190.
71. Liao CJ, Li PT, Lee YC, Li SH, Chu ST":Lipocalin 2 induces the epithelial-mesenchymal transition in stressed endometrial epithelial cells: possible correlation with endometriosis development in a mouse model. Reproduction. 2013; 20; 147(2):179-187.
72. Fried SK, Greenberg AS: Lipocalin 2: a "sexy" adipokine that regulates 17beta-estradiol and obesity. Endocrinology. 2012; 153(4): 1582-1584.
73. Voronenko NY: Adipokines resistin and lipokalin-2 and its role in the pathogenesis of polycystic ovary syndrome and metabolic syndrome. Medičnì Perspektivi. 2013; 18(3) 95-110.
74. Liao SL, Chen WY, Chen CJ: Estrogen attenuates tumor necrosis factor-alpha expression to provide ischemic neuroprotection in female rats. Neurosci Lett. 2002; 20; 330(2):159-162.
75. Muthukumar S, Rajesh D, Saibaba G, Alagesan A, Rengarajan RL, Archunan G: Urinary lipocalin protein in a female rodent with correlation to phases in the estrous cycle: an experimental study accompanied by in silico analysis. PLoS One. 2013; 14; 8(8):e71357.
76. Roudkenar MH HR, Ghasemipour Z, Roushandeh AM, Rouhbakhsh M, Nekogoftar M, Kuwahara Y, Fukumoto M, Shokrgozar MA: Neutrophil gelatinase-associated lipocalin acts as a protective factor against H(2)O(2) toxicity. Arch Med Res. 2008; 39:560–566.
77. Halabian R, Tehrani HA, Jahanian-Najafabadi A, Habibi Roudkenar M: Lipocalin-2-mediated upregulation of various antioxidants and growth factors protects bone marrow-derived mesenchymal stem cells against unfavorable microenvironments. Cell Stress Chaperones. 2013; 18(6):785-800.
78. Balistreri CR, Caruso C, Candore G: The role of adipose tissue and adipokines in obesity-related inflammatory diseases. Mediators Inflamm. 2010; 2010:802078. doi: 10.1155/2010/802078.
79. Alwahsh SM, Xu M, Seyhan HA, Ahmad S, Mihm S, Ramadori G, Schultze FC: Diet high in fructose leads to an overexpression of lipocalin-2 in rat fatty liver. World J Gastroenterol. 2014; 21; 20(7):1807-1821.