

## Fetal sex and leptin concentrations in pregnant females

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**BACKGROUND:** Adult females have significantly more leptin than males. We investigated the effect of fetal sex on levels of leptin and other pregnancy hormones (progesterone, estradiol, FSH, LH and  $\beta$ hCG) in pregnant females during different stages of pregnancy.

**PATIENTS AND METHODS:** Serum leptin levels in pregnant females followed at King Khaled University Hospital, Riyadh, Saudi Arabia in the year 2001 were estimated at the first, second, and third trimester of pregnancy as well as after delivery. Progesterone, estradiol, FSH, LH and  $\beta$ hCG levels were also measured.

**RESULTS:** Leptin levels were significantly higher during all three trimesters in the pregnant females carrying female fetuses compared with those with male fetuses, while other hormones did not differ between the two groups. After delivery, the leptin levels decreased in both groups, and were still higher in the females who delivered baby girls, but the results were not statistically significant.

**CONCLUSION:** These finding suggests that leptin might play a role in sex regulation during embryonic development.

Leptin, the product of the obese gene (*ob*) gene, is a hormone secreted by adipocytes.<sup>1</sup> It has important effects in controlling body weight, metabolism and reproductive functions.<sup>2,3</sup> In adult humans, females have significantly more leptin than males.<sup>4</sup> The significant decrease in serum leptin concentration in males reflects both the increasing concentration of muscle mass to body mass index (BMI) and the direct inhibitory effect of testosterone and dehydroxytestosterone (DHT) on leptin secretion.<sup>4,5,6,7,8</sup> In females, increasing leptin concentrations throughout puberty may reflect a gender difference in the expression of control of leptin resistance, promoting the accumulation of adipose tissue needed for reproduction, as well as the effect of estrogen and progesterone.<sup>4,7,9</sup> This study was carried out to investigate the effect of the sex of the baby on levels of leptin and other pregnancy hormones (progesterone, estradiol, FSH, LH and  $\beta$ hCG) in pregnant females during different stages of pregnancy.

### Patients and Methods

The study included 36 pregnant Saudi females regularly attending the antenatal clinic at King Khalid University Hospital. All women gave informed consent before recruitment. Age, gestational age at entry to the study, past obstetric history, and past medical history were recorded on specially designed forms. Twenty-six of the 36 pregnant females delivered in King Khalid University Hospital, Riyadh, Saudi Arabia, while the remaining 10 pregnant females delivered in other hospitals. Nine of the 36 pregnant females delivered female babies, while 27 pregnant females delivered male babies. The gestational age of the pregnant females was calculated from the first day of the last menstrual period and was confirmed by mid-trimester ultrasound scan. The height of the female was measured at entry to prenatal care, and was followed until delivery. Maternal weight was measured at each visit. Body mass index (BMI) was calculated by dividing the weight in kilograms by the height in meters squared. Fetal growth was evaluated clinically and by ultrasound. Outcome of pregnancy, mode of delivery, and sex of the baby, length, and weight were recorded.

Blood samples for estimation of leptin concentrations were obtained from the pregnant females at the first trimester, second trimester, third trimester and postnatally (on

#### Abbreviations

$\beta$ hCG beta human chorionic gonadotropin

BMI body mass index

DHT dehydroxytestosterone

E2 estradiol

FSH follicle-stimulating hormone

LH luteinizing hormone

LHRH luteinizing hormone-releasing hormone

*ob* gene obese gene

RIA radioimmunoassay

the second day after spontaneous vertex delivery, and on the third day after caesarian section). The collection of blood samples was between 8 AM and 5 PM without overnight fasting. Blood samples (5 mL) were drawn by venipuncture in plain tubes (red top tubes). The blood was allowed to clot and the serum was separated by centrifuge at 4000 rpm for 7 minutes at room temperature and was stored frozen at -20°C until required for analysis. Serum leptin concentrations were estimated in duplicate by a human leptin radioimmunoassay (Linco Research, St Charles, MO, USA). The within-batch coefficient of variation (CV) for leptin ranged from 1.6% to 13.0%, while the between-batch CV was 3.9%. Serum progesterone, E2, FSH, LH and βhCG concentrations were estimated in duplicate by autoanalyzer (Elecsys 2010, Roche, Immunoassay System). For progesterone the within-batch CV ranged from 0.35% to 2.67%, while the between-batch CV was 10.9%. For E2 the within-batch CV ranged from 0.05% to 1.86%, while the between-batch CV was 10.4%. For βhCG the within-batch CV ranged from 0.29% to 4.08% while the between-batch CV was 12.4%.

Data analysis was carried out using SPSS program version 10. Data are presented as the mean±SD. Student's t test was used to determine the significance of the difference of the means in any two trimesters and after delivery. A P value <0.05 was considered statistically significant.

**Results**

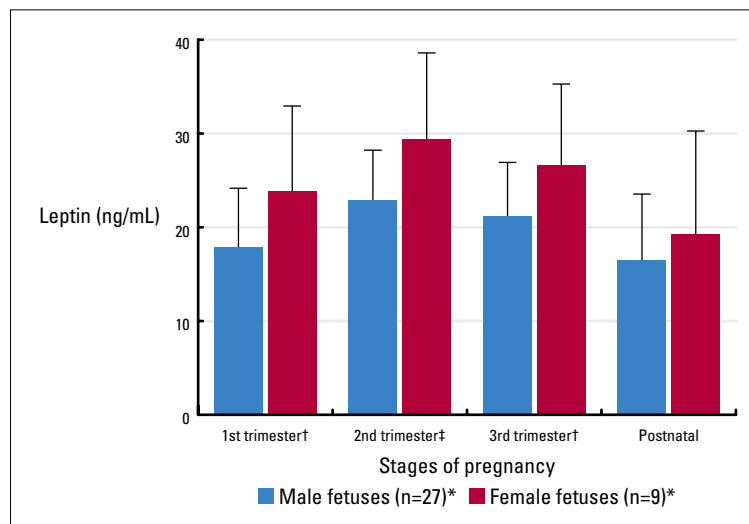
In the comparison of mothers according to the sex of the fetus, maternal age and BMI did not differ between the two groups, but placental weight and BMI were higher in women pregnant with male fetuses compared to women with female fetuses (Table 1). The mean maternal leptin levels were significantly higher during all three trimesters in women with female fetuses compared to women with male fetuses and the difference was statistically significant (Figure 1). However, after delivery leptin levels decreased in both groups and were still higher in females who delivered baby girls, but the results were not significant statistically.

Pregnant females with female fetuses had higher mean progesterone levels during the three trimesters compared with pregnant females with male fetuses, but the difference was not statistically significant (P>0.05) (Figure 2). After delivery progesterone levels decreased significantly in both groups, but the difference between the two groups was not

**Table 1.** Demographic characteristics of pregnant women according to fetal sex.

Variables	Pregnancies with female fetus	Pregnancies with male fetus
Number	9	27
Maternal age (y)	29±10	28±12
Maternal height (m)	1.64±0.21	1.65±0.19
Maternal weight (kg)	68.09±10.3	69.56±11.42
Maternal BMI after delivery	27.98±5.6	28.56 ±4.33
Placenta Weight (g)	526.67±68.9	599.69±149.34
Neonatal Height (cm)	48.34±2.8	49.15±2.8
Neonatal weight (kg)	3.25±0.54	3.26±0.63
Neonatal BMI (kg/m <sup>2</sup> )	13.8±1.56	13.44 ±1.9

**Figure 1.** Mean (±SD) concentrations of leptin in pregnant women by the sex of the fetus.

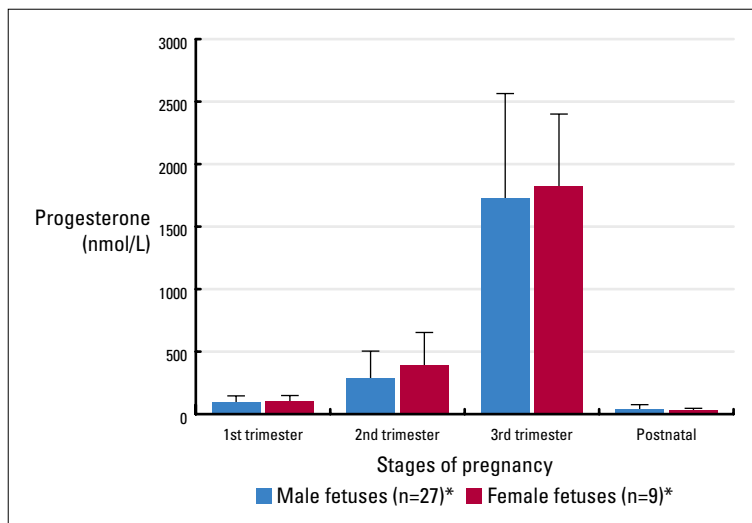


\* For postnatal period, n=18 for male fetuses and n=8 for female fetuses

† P<0.05 male fetuses vs. females fetuses

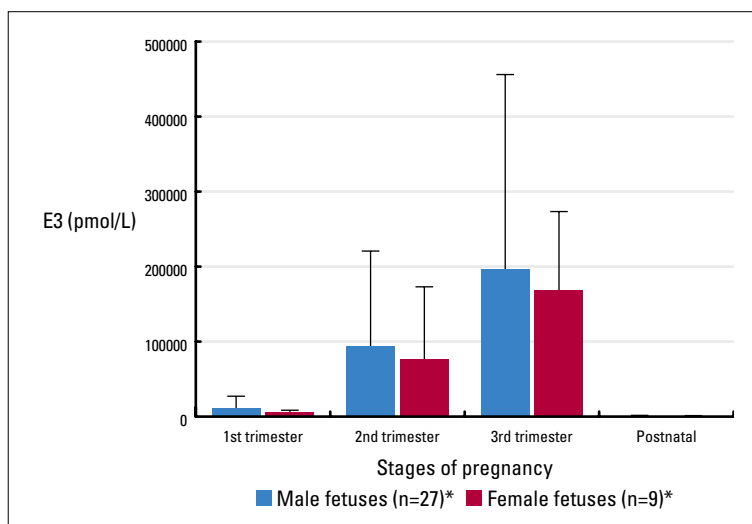
‡ P<0.05 males fetuses vs. females fetuses

**Figure 2.** Mean ( $\pm$ SD) concentrations of progesterone in pregnant women by the sex of the fetus.



\* For postnatal period, n=18 for male fetuses and n=8 for female fetuses

**Figure 3.** Mean ( $\pm$ SD) concentrations of estradiol in pregnant women by the sex of the fetus.



\* For postnatal period, n=18 for male fetuses and n=8 for female fetuses

significant statistically. The mean values for E2 were higher in pregnant females with male fetuses compared with pregnant females with female fetuses, but the difference was not statistically significant ( $P>0.05$ ) (Figure 3). During the first trimester pregnant females with female fetuses had higher mean  $\beta$ hCG levels compared with females who delivered male babies, but there was no statistically significant difference ( $P>0.05$ ) (Figure 4). However, during all other stages of pregnancy females who delivered male fetuses had slightly higher mean  $\beta$ hCG levels, but with no statistically significant difference ( $P>0.05$ ). Similarly, there were no differences in maternal age, maternal BMI, placental weight, baby BMI and gestational age between the two groups.

### Discussion

Leptin, the adipocyte hormone, has been the subject of considerable investigation during the last decade due to its possible role in the regulation of body weight and reproductive functions.<sup>1,2,3</sup> Generally, females have higher leptin levels compared to males,<sup>4</sup> and leptin levels were shown to correlate with the amount of body fat in non-pregnant subjects. Few studies have been carried out on pregnant females, even though many reports showed elevated maternal serum leptin levels during pregnancy, compared to the leptin levels in non-pregnant women.<sup>10,11,12,13</sup> In the present study, we investigated leptin levels during the first, second and third trimesters of pregnancy in relation to other pregnancy hormones, maternal weight, BMI and the sex of the babies. In addition, we also investigated leptin levels after delivery and correlated leptin and the different pregnancy hormones, maternal and babies weight and BMI, and placental weight. In this respect, our study is unique, as no other study has been conducted with the same detailed objectives.

In the pregnant females, serum leptin levels were lowest during the first trimester, but increased significantly from the first trimester to the third trimester, while a slight but insignificant decrease occurred during the third trimester. After delivery, plasma leptin levels decreased significantly, reaching almost the non-pregnant levels within a few days after delivery. These results confirm the previous reports by Hardiel et al<sup>10</sup> and Sivan et al,<sup>11</sup> who reported elevated leptin levels during pregnancy and a significant decrease after delivery.

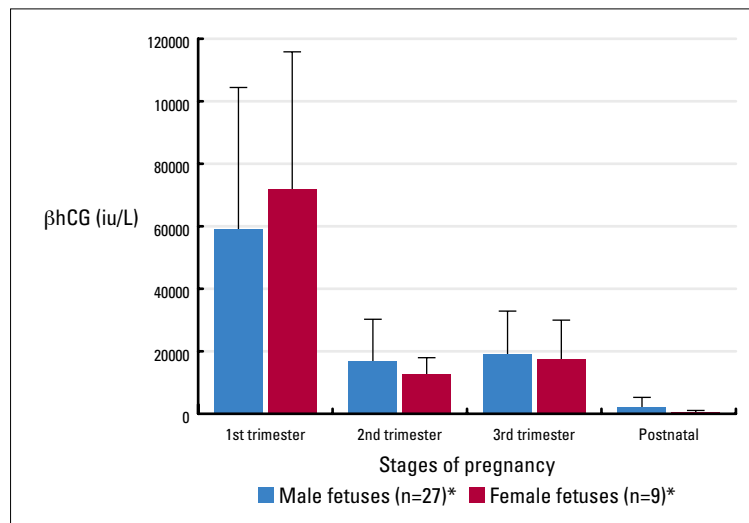
$\beta$ hCG levels reached a peak value during the first trimester and from the first trimester to the second trimester there was a significant decrease in

concentration, while during the third trimester there was slight increase in serum  $\beta$ hCG concentrations, which was not significant. After delivery, a sharp decrease occurred in serum  $\beta$ hCG concentrations. Progesterone and E2 levels increased continuously throughout pregnancy until they reached a peak value during the third trimester. After delivery there was a significant decrease in both serum progesterone and E2 levels.

To investigate the effect of the fetal sex on leptin levels and other pregnancy hormones we separated the results obtained during the first, second and third trimesters and after delivery by the sex of the babies. We compared these results during each trimester between the females who had male babies and those who had female babies. Our finding showed an interesting difference throughout pregnancy where there was a significantly higher level of serum leptin in pregnancies with female fetuses compared with pregnancies with male fetuses. Our results are in agreement with those of Helland et al<sup>14</sup> who showed a higher leptin in maternal serum of females carrying female fetuses compared with those carrying male fetuses.

Other pregnancy hormones ( $\beta$ hCG, progesterone and E2) showed no differences in levels between the two groups. Similarly, there were no differences in maternal age, maternal BMI, placental weight, baby BMI and gestational age between the two groups. These results suggest that the gender difference in serum leptin concentrations are present from early pregnancy and the increase in serum leptin concentrations seen in the mothers carrying female infants must be independent of reproductive hormones, since at different stages of pregnancy maternal serum showed no differences in the pregnancy hormones according to the sex of the fetuses. Similarly, this difference in leptin levels between the two groups is unlikely to be due either to the body fat distribution or to reproductive hormone status, as these were shown to be not different in male and female fetuses. Interestingly, these gender differences during pregnancy were not present after delivery, and the leptin levels were similar in both groups of

**Figure 4.** Mean ( $\pm$ SD) concentrations of [beta]hCG in pregnant women by the sex of the fetus.



\* For postnatal period, n=18 for male fetuses and n=8 for female fetuses

women. These results are in contrast to other investigations, which showed an elevation of leptin level in mothers delivering female babies compared with those delivering male babies.

Our results show that serum leptin levels increase from the first trimester to the second trimester, but beyond this they decrease slightly but insignificantly during the third trimester. After delivery, a significant decrease in leptin levels takes place and within a few days postnatally the non-pregnant levels are reached. Furthermore, we observed that pregnant mothers with female fetuses had higher leptin concentrations than pregnant mothers with male fetuses while there was no difference in serum progesterone, E2, and  $\beta$ hCG concentrations between the two groups. These findings suggest that leptin may play a role in sex regulation during early embryonic development, although further studies are needed to evaluate other possible roles of leptin in embryonic development.

### Acknowledgements

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