Morphometric study of kidney and suprarenal gland development after super ovulation injection in mice

Mohammad O. Selman FIBMS, Ban T. Al-Ani MSc, Rana R. Al-Saadi MSc and Usama I. M. AL-Zubaidi MSc
Department of Applies Embryology, High Institute for Infertility Diagnosis and Assisted Reproductive Technology-Al-Nahrain University Correspondence Author e-mail: mohammadoda@yahoo. com

Abstract:

Background:
Superovulation in mice causes a delayed embryonic development *in vitro* and in vivo, an increased abnormal blastocyst formation, pronounced fetal growth retardation.

Objective:
Morphometric measurements the dimensions (thickness, length) of fetal kidney and suprarenal gland during the later intrauterine life, after superovulation induction.

Methods:
Twenty five mature female mice Swiss-Webster mice, these mice were divided into two groups: experimental (G1) (15animal/group) and control group (G2) (10 animal/group). Superovulation was induced by intraperitoneal (IP) injection of 7.5 IU of PMSG, then followed by 7.5 IU of HCG after 48 hours. Ovulation was taken place between 12 ±2 hours after injection of HCG. One mature male mice was placed with three superovulated female mice for mating. Pregnant females were sacrificed on gestational day eighteen in all experimental groups. For morphometric study 20 mice embryos were fixed in Bouin>s fixative, longitudinal sections of whole embedded embryo were stained by H&E, the specimens independently read. The dimensions (thickness, length) of fetal kidney and suprarenal gland were evaluated.

Results:
The statistical analysis showed significant decrease (P< 0.01) in weights of fetuses in comparison with control group. The study was showed significant decrease (P< 0.01) in fetal kidney dimensions at day 18 of intrauterine life, when it compared to the fetal kidney of non superovulated group (control group). There was also significant decrease (P< 0.01) in the morphometric measurements of fetal suprarenal gland dimensions.

Conclusion:
The study improve that there is a delay in growth of kidney and suprarenal gland in fetal mice that belongs to mother undergo to superovulation induction before pregnancy.

Key word: Superovulation, Suprarenal gland, Morphometry

Introduction:

Superovulation is routinely used in both animals and humans to induce multiple follicle stimulation thus increasing the number of oocytes or embryos obtained from a single cycle that can potentially be used for techniques such as *in vitro* fertilization (IVF). The use of exogenous gonadotropins to induce ovarian stimulation, however, has been reported [1, 2, 3]. Furthermore, ovarian stimulation was associated with a reduction in fetal growth and a prolonged gestation period in mice [4]. Superovulation in the mice causes a delayed embryonic development *in vitro* and in vivo, an increased abnormal blastocyst formation, pronounced fetal growth retardation, and an increased number of resorption sites [5]. Developmental delay and growth restriction have also been observed following superovulation. When embryos were cultured from the blastocyst stage, development was delayed relative to controls (6). Growth and development of the embryo during the embryonic stage of pregnancy are influenced by uterine factors (7). Embryologically, urinary system develop from a common mesodermal ridge (intermediate mesoderm) along the posterior wall of the abdominal cavity, In human, the permanent kidney (metanephros) appears in the fifth week of gestation, the kidney develops from two sources metanephric mesoderm, which provides excretory units; and the ureteric bud, which give rise to the collecting system [8].

Development of the human fetal kidney runs through a series of continual and mutually dependent changes during which the kidney obtains its morphological and functional maturity [9]. Fetal kidneys do not reach the same level as adults at full term and move farther apart from the midline of the body during the fetal period, the dimensions, weight, and volume of the kidneys increased with gestational age during the fetal period [10]. Suprarenal gland which is structurally and functionally unique from other species suprarenal gland plays a pivotal role, mainly through steroidogenesis, in the regulation of intrauterine homeostasis and in fetal development and maturation. The steroidogenic activity is characterized by early transient cortisol biosynthesis, followed by its suppressed synthesis until late gestation, and extensive production of dehydroepiandrosterone and its sulfate, precursors of placental estrogen, during most of gestation [11].

Materials and Methods:

All experiments were performed on 25 mature female Swiss-Webster mice, their ages ranged between 8-12 weeks with a body weight ranged between 25-30 g. Mice were obtained from the colony of the animal house of the Institute of Embryo Research and Infertility Treatment, Al-Nahrain University/Baghdad. These mice were divided into two groups: one experimental (G1) (15 animal/group) and control group (G2) (10 animal/group). Induce ovulation in experimental group (G1) was done by i. p. injection of 7.5 IU of pregnant mare serum gonadotropin (PMSG), then followed by i. p. injection of 7.5 IU of human chorionic gonadotropin (HCG) after 48 hours, ovulation was taken place between 12±2 hours after injection of HCG, then after 6 hours of HCG injection, one mature healthy male mice was placed with three superovulated female mice for mating, then check for successful mating by looking for the presence of vaginal plug, The presence of vaginal plug indicated successful mating and this was designated day 1 of gestation, fertilization usually complete by 6 hours after mating [12]. Control group (G2) were injected with the vehicle at the appropriate time. When the pregnant females of each group (G1 and G2) reached day 18 of gestation they were sacrificed by cervical dislocation, each fetus was examined for weighed then washed and fixed in Bouin>s solution for 24 hours, then stored in 70% ethanol alcohol for routine histological techniques, paraffin sections with 5 microns thickness were prepared and stained with Harries hematoxylen-eosin stain for histological examination [13]. Serial measurements of kidney and adrenal gland dimensions (length, width) were performed in 20 mice fetuses'. For morphometric analysis, images were captured by using Microns Contain TV-Based computer assisted morphometry with a 10X and 4 X objectives. The actual measurements were done by using the image analyzer software after accurate calibration using a stage micrometer.

The morphometric parameters used in this study:

- Using Microsoft (Motic image plus 2.0) for measured the length and diameter from menu chose open to open this image, then from measure chose objective (10X) or (4X), and unit that using to measure (mm), and then chose from menu irregular shape or line to draw, the area tend from measurement table that found in menu program (software) and then storage this images in computer.
- Because irregular shape for these images, and after measured the area, for measured the diameter for this parameters, this process is called D-circle.

Results:

A- Weights changes:

The statistical analysis showed significant decrease (P< 0.01) in embryos weights (males and females) belonged to mothers treated with normal dose of PMSG at day 18 in comparison with control group figure (1).

B. Morphmetric Measurement:

Mean values were calculated from the treated group with PMSG and non treated group during day 18, the morphometric measurement of the kidney length of treated group was 281.690 µm, while the morphometric measurement of kidney length of non treated group (control group) was 431.095 µm. So there is significant decrease in kidney length when it compared with non treated group.

The result of morphometric measurement of kidney thickness of treated group was 210 µm, and the result of morphmetric measurement of non treated group (control group) was 269.8 µm um. So there is significant decrease in kidney width when it compared with non treated group figure (2).

The morphometric measurement of suprarenal gland thickness of treated group was 95.6 µm, while of non treated group (control group) was 127.4 µm. The morphmetric measurement of suprarenal gland length of treated group was 98.9 µm, while of non treated group (control group) was 129.8 µm. So there is significant decrease in suprarenal length and thickness when it compared with non treated group figure (3).

Longitudinal section of mice fetus at day 18 belongs to mothers injected by IP of 7.5 IU of PMSG, followed by 7.5 IU of HCG morphometric study showing the kidneys (metanephron) adjacent to the adrenal gland in the abdominal cavity as shown in figure (4).

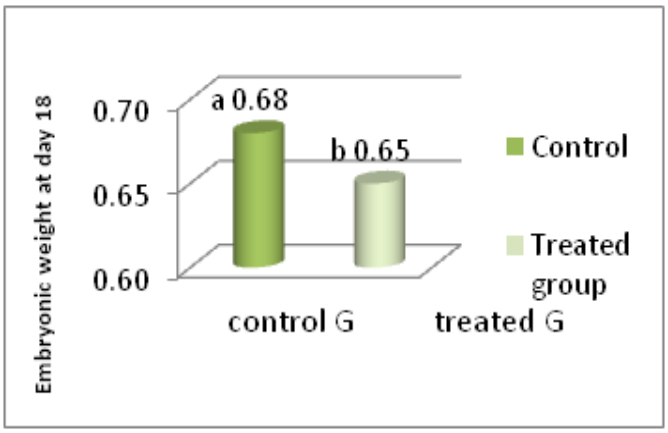

Figure (1): A diagram showing the differences in embryos weight belong to mothers treated with PMSG and non treated groups (control group) at day 18.

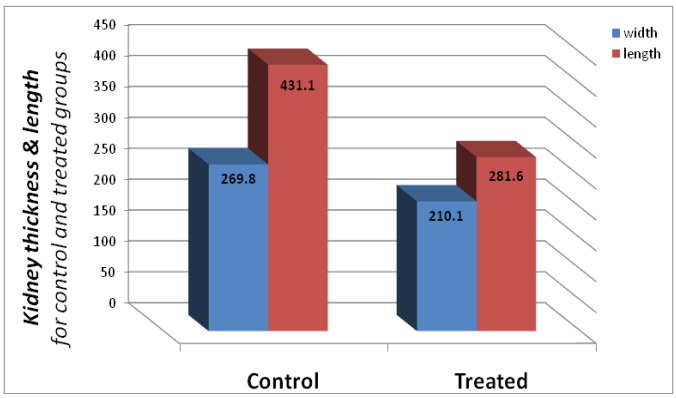

Figure (2): A diagram showing the differences in the kidney length and thickness during prenatal life between treated groups and non treated group (control group).

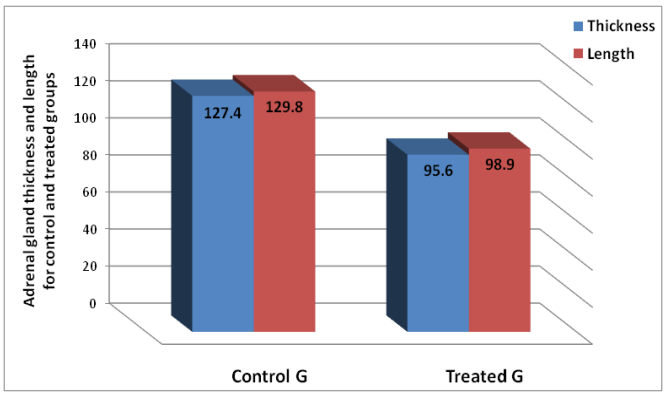

Figure (3): A diagram showing the differences in the adrenal gland length and width during prenatal life between treated groups and none treated group (control group).

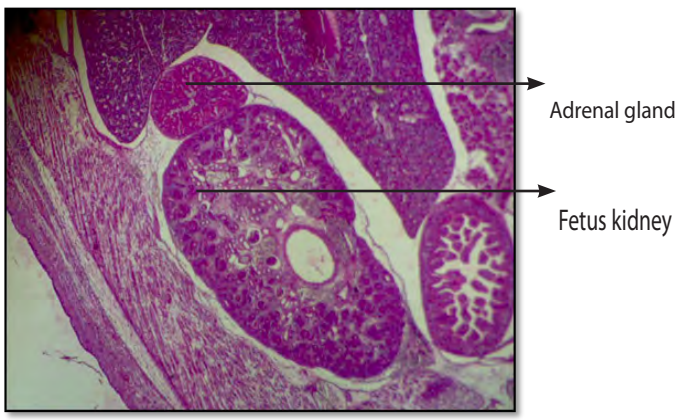

Figure (4): Longitudinal section of mice fetus at day 18 belongs to mothers injected by IP of 7.5 IU of PMSG, followed by 7.5 IU of HCG morphometric study showing the kidneys (metanephron) adjacent to the adrenal gland in the abdominal cavity (10X).

Discussions:

The kidney is one of the organs that continue to develop postnatal, kidney development does not complete during intrauterine life. Maturation of the cortico-medullay regions of the kidney continues throughout the first week of neonatal life. The rat glomeruli and tubules continued to develop and mature during that period [14]. Decreased embryos weight belongs to superovulated mother relative to

controls have been observed at E7.5 [15], E14.5 [16,17], E18.5 [1] and E19.5 [18].

Ovarian stimulation has also been reported to delay the in vivo development of embryos [6,16] compared with embryos recovered from naturally cycling control mice, and this has been attributed to an adverse effect of stimulation on the oocytes or embryos as well as the reproductive tract. In particular, several studies have shown that superovulation results in embryonic growth restriction [6, 16, 18] at mid- to late-gestation. Although few studies followed development through to birth, one study has also noted delayed parturition following superovulation. Similarly, implantation was delayed following superovulation in vivo [15].

Suprarenal, glands are located on the top of each kidney, these glands produce hormones that we can>t live without, including sex hormones and cortisol, which helps to respond to stress and has many other functions. The role of the fetal adrenal cortex in human pregnancy and parturition appears highly complex, probably due to redundant and compensatory mechanisms regulating these events [11].

According to Ozgüner, 2012 [19] who study the morphometric development of the suprarenal gland using anatomic dissection methods during the fetal period found that all parameters increase with gestational age, and concluded that there was significant correlation between gestational age and all parameters and found that no significant differences were observed between sexes for any of the parameters and observed that there was no difference between the right and left sides of parameters except the thickness of the suprarenal glands.

According to Vlajković S *et al*. 2006 [9] on their observation concluded that development of the human fetal kidney runs through a series of continual and mutually dependent changes during which the kidney obtains its morphological and functional maturity. Their study estimates the changes in kidney size during gestation in fetuses from the 4th to the 10th month. Serial measurements of kidney dimensions (width, thickness) were performed in 110 fetuses. The importance of this study lies in determining the average fetal kidney dimensions, which could be used as standard values in obstetrics [9,20]. These findings are in agreement with the current investigation of the anatomical morphological feature of the kidney.

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