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Research Article

Maternal serum progesterone levels and placental expression of progesterone receptors in insulin-resistant pregnant rats

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Keywords:
Insulin resistance, placenta receptors, progesterone, oestradiol, pregnancy

ABSTRACT

Background: Maternal diabetes is known to impair placental function; however, its effect on placental expression of progesterone/oestrogen receptors and role of the placenta in foetal programming has not been well documented. The study assessed serum progesterone and oestradiol levels, placental morphology, placental progesterone and oestrogen receptors expression in insulin resistant rats. **Methods:** Virgin female rats were randomly divided into 2 groups; control group fed normal rat chow and insulin resistant group fed a diet containing 25% fructose w/w orally for 12 weeks. Rats in both groups were mated with proven male rats and presence of sperm cells in vaginal smear the following morning was taken as day 1 of pregnancy. Maternal blood and amniotic fluid samples were obtained and assessed for glucose, insulin, C-peptide, progesterone and oestradiol levels. Quantitative insulin sensitivity check index (QUICKI) was estimated from fasting blood glucose and insulin values. Placental tissues were isolated, weighed and fixed for morphological studies and the expression of oestrogen and progesterone receptors using immunohistochemical technique. **Results:** Maternal blood and amniotic fluid glucose, insulin, C-peptide levels, placental weight and diameter were significantly increased; while QUICKI was significantly lower ($P < 0.05$) in the insulin resistant rats compared to control rats. Placental junctional zones were enlarged due to an increase in the number of glycogen and trophoblast giant cells in the insulin resistant rats. Progesterone receptor expression was down regulated, with no significant difference in oestrogen receptor expression in the insulin resistant rat placentae. **Conclusion:** The results suggest that maternal insulin resistance impairs progesterone production, placental morphology and down regulates placental progesterone receptor expression in this animal model of type 2 diabetes mellitus.

INTRODUCTION

Diabetes is a common metabolic disorder of pregnancy with a global prevalence ranging from 2% to 14% depending on the population studied. Gestational diabetes mellitus (GDM) and type 2 diabetes mellitus (T2D), constitute over 90% of diabetic pregnant cases (Al-Noaemi and Shalayel, 2011; Vambergue and Fajardy, 2011). Foetal exposure to a compromised intra-uterine environment impairs foetal growth and metabolic processes with long term consequences for the offspring's health, a phenomenon termed foetal programming (Barker, 1998; Armitage *et al.*, 2005). However, the mechanisms through which the offspring of diabetic mothers are programmed for metabolic disorders in later life, have not been fully documented.

Pregnancy induces metabolic changes in maternal circulation, due to pregnancy hormones, which is essential to meet energy demands of the foetus. These changes increase resistance to insulin, as most pregnancy hormones are insulin antagonist, thus enhancing glucose availability and supply to the developing foetus (Barbour *et al.*, 2007). Pancreatic beta cells however compensate for the increased demand for insulin, and a normoglycemic state is maintained. However, women who develop GDM exhibit a defect in insulin secretion, increased hepatic glucose production and resistance to insulin action, all of which contribute

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to the development of overt hyperglycaemia (Kuhl, 1991).

Although the precise mechanisms by which pregnancy hormones interact with pancreatic beta cells to produce glucose intolerance during pregnancy are uncertain, it has been suggested that the final common pathway responsible for the development of GDM is an incomplete compensation of pancreatic beta cells to increase insulin secretion in proportion to elevated circulating glucose levels. This results in the manifestation of hyperglycaemia, hyperinsulinemia, impaired glucose tolerance and gestational diabetes mellitus (Kahn, 1998).

In recent years, scientists have recognized that genetic factors are unable to fully explain the development of GDM. Increased dietary intake of food, drinks and beverages high in sugars, have been suggested to directly promote glucose intolerance during pregnancy. Consumption of such diets adversely affects energy metabolism causing hyperglycaemia and insulin resistance (Al-Noaemi and Shalayel, 2011). Fructose is an isomer of glucose and its free form is produced commercially through the isomerization of glucose. Fructose is commonly used as sweetener in processed foods, drinks and beverages.

High influx of fructose to the liver, the main organ for its metabolism, perturbs glucose uptake. This occur due to increased glycerol and acyl portions of triglyceride molecules from fructose metabolism (Basciano *et al.,* 2005). Fructose by–passes the rate limiting step of glucose metabolism, providing an unregulated amount of lipogenic substrates for conversion to fatty acids and triglyceride, as well as upregulating transcription factors and enzymes involved in lipogenesis. Increased stimulation of insulin production occurs after fructose consumption, resulting in a decreased activation of adipose tissue lipoprotein lipase (Sloboda *et al.,* 2014). Fructose therefore acts by impairing glucose disposal leading to a compensatory hyperinsulinaemia. A reversible hyperglycaemia and hyperinsulinemia (type 2 diabetes mellitus) can therefore be induced by chronic fructose feeding in animal models (Suga *et al.*, 2000; Basciano *et al.,* 2005; Arikawe *et al.,* 2008; Iranloye *et al.,* 2011).

The placenta function to provide endocrine secretions and selective exchange of substances through its apposition to both uterine and trophoblastic vasculature. Owing to its position, the functions of the placenta can be impaired by adverse maternal conditions such as diabetes mellitus (Jansson and Powell, 2007; Sferuzzi-Perri and Camm, 2016), which may occur via modulations in the expression of hormone receptors and substrate transporters on placental surfaces (Hiden and Desoye, 2010). Placental hormones are secreted mainly by the syncytiotrophoblast in a highly regulated manner. The hormones are important for the maintenance of pregnancy, exerting autocrine and paracrine effects that regulate decidualization, placental development, angiogenesis, endometrial receptivity, embryo implantation, immune-tolerance and foetal development (Costa, 2015).

Progesterone is a steroid hormone crucial for the maintenance of pregnancy; hence it is also called the 'hormone of pregnancy'. The corpus luteum is the main source of progesterone during the first weeks of pregnancy, owing to human chorionic gonadotropin (hCG) stimulation. As hCG concentration declines, the placenta gradually becomes the main source of progesterone production (Tuckey, 2005). This hormone is mainly synthesized from maternal cholesterol, through a two-step reaction occurring in the syncytiotrophoblast mitochondria.

Maternal blood levels of progesterone increase throughout pregnancy, peaking during the last 4 weeks of gestation and decreasing after labour and placental delivery. Progesterone exerts genomic and non-genomic actions, by activating its receptors. The classic nuclear progesterone receptors (PR), PRα and PRβ, are ubiquitously expressed in the female reproductive tract and the placenta, they dimerize after progesterone binding, mediating its genomic action by binding to specific DNA elements in the promotor of target genes. In addition, PR may also mediate cytoplasmic signalling pathways (Carlos *et al.,* 2013).

In addition to placentation, progesterone prepares the mammary gland for lactation by enhancing the proliferation of mammary epithelium, but prevents lactation until labour, antagonizing the prolactin effect (Pang and Hartmann, 2007). Progesterone has been reported to be involved in metabolic changes during pregnancy, promoting hyperphagia, fat storage and insulin resistance E2 (Butte, 2000). The effect of progesterone, mediated by its receptors identified in the placenta aside the uterus, suggests that the placenta itself is a target tissue for progesterone action (Tsai and O'Malley, 1994; Shanker and Rao, 1999).

Oestrogens are produced primarily by the ovaries in the non-pregnant state and by the placenta, during pregnancy. Placental oestrogens are a group of four different steroid hormones: oestrone (E1), 17β-oestradiol (), estriol (E3) and oestetrol (E4). During the first weeks of gestation, the oestrogens are produced by the corpus luteum, after which the placenta takes over the synthesis of these steroids. Blood levels of all oestrogens increase throughout pregnancy, peaking at term. Oestradiol is the most abundant oestrogen and has

been reported to inhibit lipolysis, promote hyperlipidaemia (Butte, 2000) and the development of physiological insulin resistance of pregnancy (Gonzalez *et al.*, 2000; Gonzalez *et al.*, 2002; Barros *et al.*, 2009). The actions of oestrogens are mediated by oestrogen receptors (ER), which are proteins activated when bond to oestrogens. Little is however known about the effect of maternal insulin resistance on placental expression of oestrogen and progesterone receptors.

MATERIALS AND METHODS

Animals

Sixteen female Sprague-Dawley rats aged 6 weeks, weighing 110 - 120 g were obtained from the Laboratory Animal Department, and ethical approval obtained from the College of Medicine, University of Lagos, Lagos. Guidelines with the use and care of laboratory animals were strictly adhered to. The animals were housed in clear polypropylene cages lined with wood chip beddings. Animals were kept under standard conditions of temperature 27ºC - 30ºC, with 12h light/dark cycle and were randomly divided into 2 groups. Group 1 served as the control group and was fed with standard rat chow. Group 2 served as the insulin resistant (IR) group and was fed *ad libitum* on a special diet containing 25% fructose (SIGMA Aldrich, USA) mixed with 75% normal rat chow weight/weight for 12 weeks (Arikawe *et al.*, 2013). At this fructose concentration, insulin resistance state was 100% achieved with zero mortality rate.

Body weight and fasting blood glucose levels were measured at the beginning of the experiment and weekly from the tail vein of the rats in both groups using Dextrostix Test Strips (Bayer Corporation, U. K.) after an overnight fast till diabetes was induced in the IR rats. Hyperglycaemia was confirmed using the glucose oxidase method (Hugget and Nixon, 1957). All animals had free access to drinking water throughout the duration of the study. The procedures were performed in accordance with guidelines of the College Ethical Committee on the use of laboratory animals for research.

Vaginal smears and Induction of pregnancy

The stages of estrous cycle in control and insulin resistant groups was determined by microscopic analysis of the predominant cell type in vaginal smears obtained daily from the 10^{th} week till the 12^{th} week of feeding i.e. a two-week period (Marcondes, *et al*., 2002). Only female rats showing two consecutive estrous cycles of the same length were used (Cruz, *et al*., 1990). Pregnancy was induced at the end of the 12^{th} week of fructose feeding by mating at night a pro-estrous female rat with mature and proven adult male rat at ratio 2:1. This was to ensure that copulation occurred at estrus, the only time the female rat is receptive to the male rat. Successful mating was confirmed by the presence of sperm cells in the vaginal smear and was regarded as day 1 of pregnancy.

Sample collection and assay of parameters

Cervical dislocation was carried out on rats in the two groups on day 19 of pregnancy. Maternal blood samples were collected by cardiac puncture into plain sample bottles, allowed to clot and centrifuged at 3,000 rpm for 15 minutes to get clear serum samples, which were subsequently kept frozen (-20ºC) until measurement of the different parameters (insulin, C-peptide, progesterone and oestradiol levels) using Enzyme-linked immunosorbent assay (ELISA) kits procured from Elabscience, FRC. Laparatomy was done and the gravid uterus exposed, clear amniotic fluid was obtained from the gestational sacs using a 28G needle and 1ml syringe.

Quantitative Insulin Sensitivity Check Index (QUICKI)

QUICKI is an index for assessing insulin sensitivity as the reference glucose clamp (Cacho *et al.*, 2008). This index was derived for maternal blood and amniotic fluid samples using fasting blood glucose and insulin levels, as the inverse log sum of glucose levels in milligram per decilitre and insulin levels in micro - unit per millilitre.

Formula: $QUICKI = 1 / [\log (I_o) + \log (G_o)]$, where I_o = fasting insulin; G_o = fasting glucose (Katz *et al.*, 2000).

Assessment of placental parameters and morphological studies

The gestational sacs were carefully opened following laparatomy and amniocentesis to isolate placental tissues. These were weighed, placental thickness and diameter were measured using a vernier caliper and the tissues fixed for morphological and immunohistological studies. Following placental tissue isolation for morphological studies, tissues were fixed in 10% formol saline, subsequently dehydrated in ascending grades of ethyl alcohol starting from 50% to two changes of absolute alcohol. Tissues were cleared in xylene, infiltrated with paraffin wax, embedded at 56^0C and sectioned at 3µm. The sections were obtained at five different non-adjacent levels through the placentae with the third level being at the approximate midpoint. Sections were mounted on glass slides and the method described by Fischer *et al*., (2008) was adopted for hematoxylin and eosin staining in the following sequence.

Immunohistochemical detection of oestrogens and progesterone receptors (ER and PR)

Paraffin embedded sections (3µm thick) were deparaffinized and rehydrated in a graded series of

alcohol. Following a rinse with phosphate buffered saline (PBS), the endogenous peroxide was blocked and the sections were incubated with 0.5 % bovine serum albumin for 30 minutes. The sections were afterwards incubated overnight with ER and PR antibodies respectively at 4°C, rinsed in PBS three times for five minutes each, followed by incubation with a biotinylated secondary antibody for 30 minutes at room temperature and for further 30 minutes with a streptavidin-peroxidase complex. Immunostaining was enhanced and visualized with 3, 3-diaminobenzidine (DAB) after sections were counterstained with Harris hematoxylin.

Quantitative immunoreactivity and image analysis

To quantify the expression of oestrogens and progesterone receptors (ER and PR) in the placental tissues, an Apex microscope was connected to a computer using an Apex Minigrab (Apex Microscopes, Wiltshire, UK). The number of golden stained nucleus per high-power field (HPF) in placenta sections from the control and insulin resistant rats were determined using an image processing program-Image J (National Institutes of Health, Bethesda, USA).

Statistical analysis

Analysis of data was done using GraphPad Prism version 6.00 for windows (GraphPad Software, San Diego, California). Results are expressed as mean ± standard error of mean (S.E.M). The significance of difference between groups were analysed using Student's t-test. Differences were considered statistically significant at $P < 0.05$.

RESULTS

Effect of fructose feeding on FBG levels in non-pregnant female rats

Fasting blood glucose (FBG) levels following fructose feeding are shown in Figure 1. FBG levels were not significantly different in the insulin resistant rats compared to control rats at the beginning of the experiment till the 4th week (insulin resistant group: initial- 79.8 ± 5.5 mg/dl, 1st week- 80.4 ± 3.4 mg/dl, 2nd week - 82.4 ± 6.0 mg/dl, 3rd week- 88.4± 4.1 mg/dl, 4th week- 89.6± 3.2 mg/dl); (control group: initial- 78.5 ± 6.3 mg/dl, 1st week- 76.7± 4.3 mg/dl, 2nd week- 80.8 ± 5.4 mg/dl, 3rd week- 82.2 ± 7.6 mg/dl, 4th week- 87.5± 6.3 mg/dl) respectively. FBG levels were however significantly increased ($P < 0.05$) from the 8th to the 12th week in the insulin resistant rats (105.2 ± 8.4 mg/dl, 120.6 ± 6.8 mg/dl, 124.9 ± 4.6 mg/dl and 130.1 ± 5.8 mg/dl) compared to control rats (84.6 ± 6.5 mg/dl, 78.8 ± 3.2 mg/dl, 80.0 ± 6.4 mg/dl and 78.1± 5.2 mg/dl) respectively. Insulin-resistant rats fed on 25% fructose w/w diet had FBG levels >120mg/dl (diabetic threshold) at the end of the 12th week of feeding.

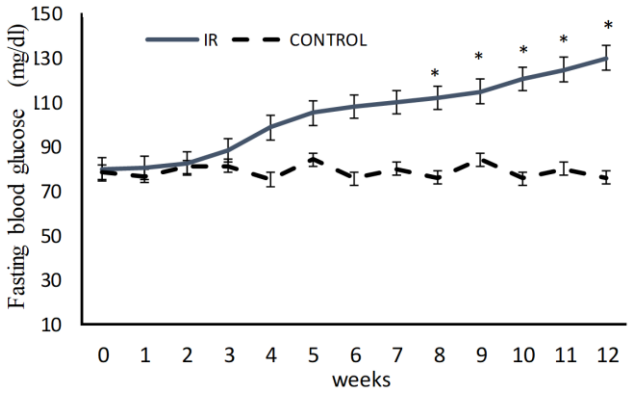

Fig. 1: Fasting blood glucose (FBG) levels of control and insulin resistant (IR) female rats following fructose feeding. FBG levels were significantly increased (*P < 0.05) in IR rats from the 8th week of feeding compared to control rats.

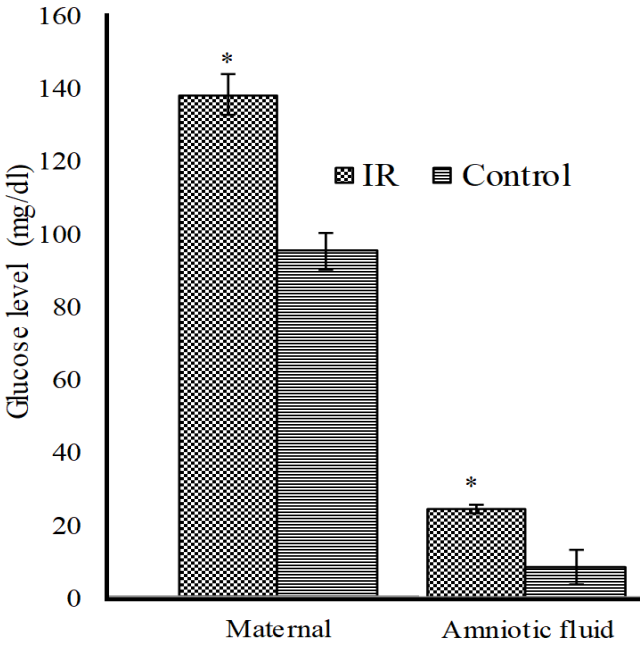

Figure 2: Maternal serum and amniotic fluid glucose levels in control and insulin resistant (IR) pregnant rats. Glucose levels in maternal serum and amniotic fluid were increased (*P < 0.05) in IR compared to control rats.

Effect of insulin resistance (IR) on maternal serum and amniotic fluid glucose levels in pregnant rats

Fasting blood glucose (FBG) levels in control and IR pregnant rats are shown in Figure 2. Maternal FBG levels in pregnant rats were significantly increased ($p < 0.05$) in IR rats (146.9 ± 9.1 mg/dl) compared to control rats (97.2 ± 4.8 mg/dl). Likewise, amniotic fluid glucose levels were significantly increased ($P < 0.05$) in IR rats compared to control pregnant rats (Figure 2).

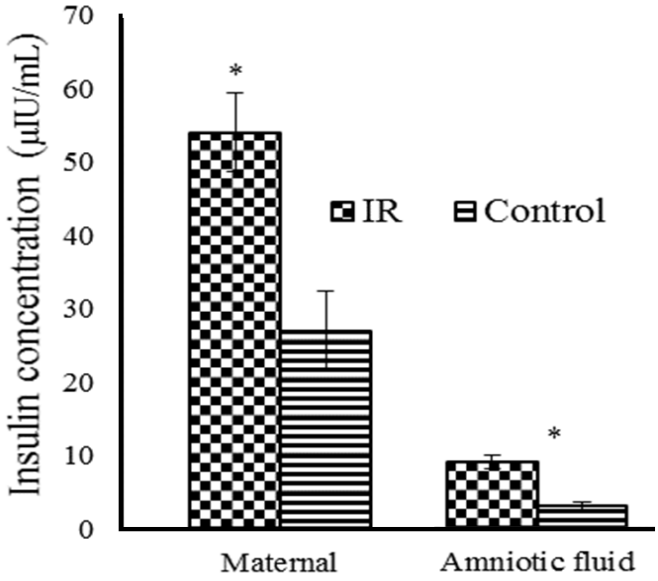

Fig. 3: Maternal serum and amniotic fluid insulin levels in control and insulin resistant (IR) pregnant rats. Insulin levels in maternal serum and amniotic fluid were increased (*P < 0.05) in IR compared to control rats.

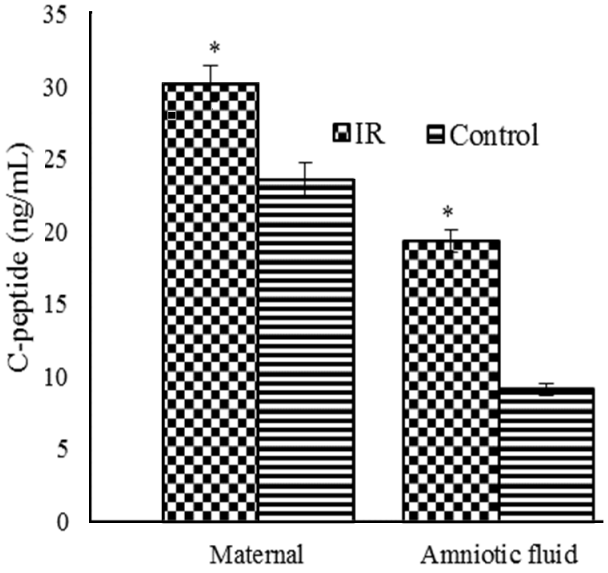

Fig. 4: Maternal serum and amniotic fluid C-peptide levels in control and insulin resistant (IR) pregnant rats. C-peptide levels in maternal serum and amniotic fluid were increased (*P < 0.05) in IR compared to control rats.

Effect of insulin resistance (IR) on maternal serum and amniotic fluid insulin levels in pregnant rats

Maternal serum and amniotic fluid insulin levels in IR and control pregnant rats are shown in Figure 3. IR rats had significantly increased (P < 0.05) insulin levels (54.1 ± 2.1µIU/mL, 8.8 ± 0.2 µIU/mL) compared to control rats (27.2 ± 0.7 µIU/mL, 3.1 ± 0.2 µIU/mL) respectively.

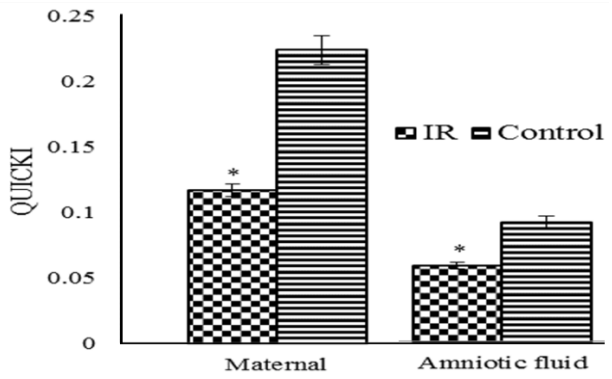

Fig. 5: QUICKI values of maternal serum and amniotic fluid in insulin resistant (IR) and control pregnant rats. A decrease (*P < 0.05) was observed in maternal serum and amniotic fluid QUICKI levels compared to Controls.

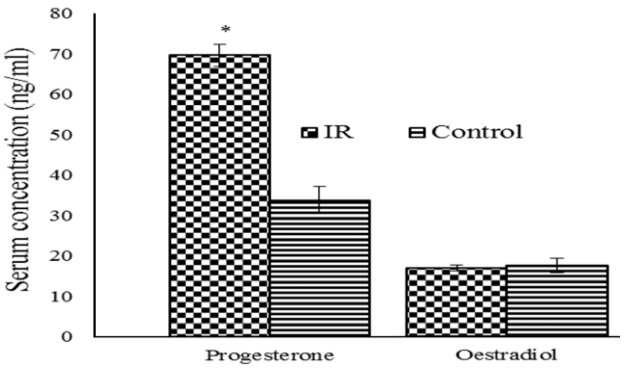

Fig. 6: Maternal serum progesterone and oestradiol levels in insulin resistant (IR) and control pregnant rats. Progesterone levels were increased (*P < 0.05) in IR rats while no significant difference was observed in oestradiol levSNSels of IR compared to control rats.

Effect of insulin resistance (IR) on maternal serum and amniotic fluid C-peptide levels in pregnant rats

C-peptide levels in maternal serum and amniotic fluid of IR and control pregnant rats are shown in Figure 4. A significant increase (P < 0.05) was observed in the maternal serum and amniotic fluid C-peptide levels of IR pregnant rats (30.2±4.3ng/ml, 19.1±3.8ng/ml) compared to control rats (23.6±2.5ng/ml, 9.1±2.2ng/ml) respectively.

Effect of insulin resistance (IR) on maternal serum and amniotic fluid QUICKI values in pregnant rats

QUICKI of maternal serum and amniotic fluid of IR and control rats, derived from maternal and amniotic fluid insulin (Io) and glucose (Go) levels as the inverse log sum of insulin in micro - unit per millilitre and glucose in milligram per decilitre, are shown in Figure 5. QUICKI of pregnant IR rats were significantly (P < 0.05) lower (0.116±0.002, 0.057±0.002) compared to control rats (0.223±0.001, 0.090±0.002) respectively.

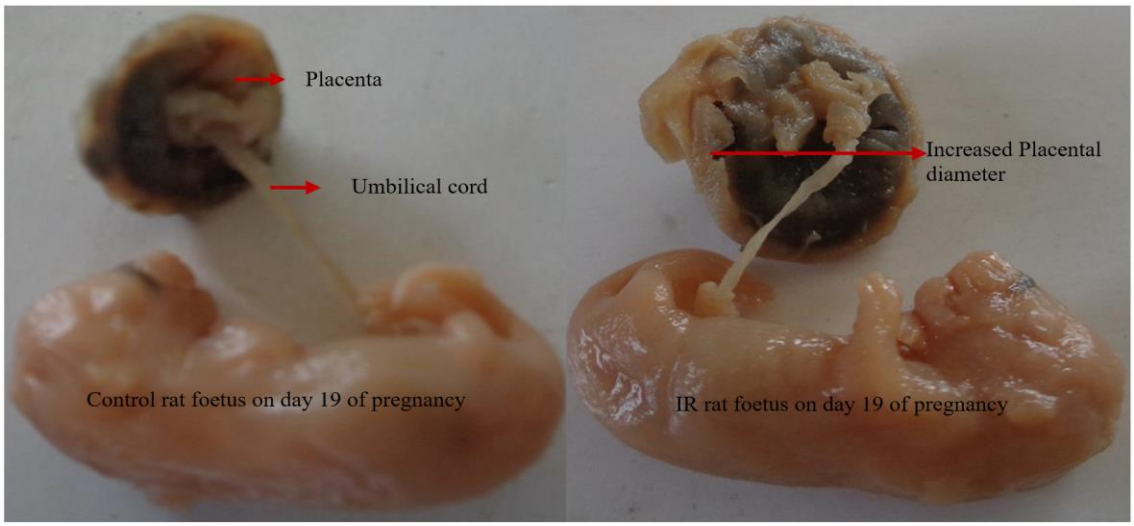

Fig. 7: Picture of Day 19 foetuses and placentae of insulin resistant (IR) and control rats. (Sony cybershot 12.1 MP Digital Camera X46). The placental diameter of IR rats was seen to be larger compared to control rats.

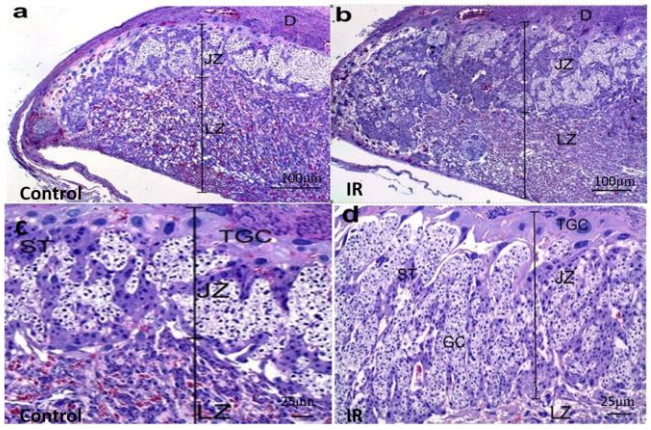

Fig. 8: (a) Photomicrographs of day 19 control rat placenta and (b) IR rat placenta showing the compartments namely decidua (D), junctional zone (JZ) and labyrinth zone (LZ). The JZ is seen as enlarged in IR placentae. H&E stain (40x) Scale bars-100µm. (c) control and (d) insulin resistant rat placenta showing the junctional zone (JZ) composed of trophoblast giant cells (TGC), spongiotrophoblast cells (ST) and glycogen-containing cells (GC) PAS stain. 100x. Scale bars- 25µm. GC is increased in the IR compared to control placentae.

Effect of insulin resistance (IR) on serum progesterone and oestradiol levels in pregnant rats

Serum progesterone levels were significantly increased ($P < 0.05$) in IR rats (69.6 ± 9.1ng/ml) compared to control rats (33.8 ± 4.6ng/ml). Serum oestradiol levels were however not significantly different in IR rats (16.9 ± 1.54ng/ml) compared to control rats (17.5 ± 1.87ng/ml) as shown in Figure 6. Picture of Day 19 foetuses and placentae from insulin resistant and control pregnant rats is presented as Figure 7 at X40 magnification.

1: Effect of insulin resistance (IR) on placental and foetal parameters in rats

Parameters	Control	IR
Placental weight (g)	0.28 ± 0.03	0.40 ± 0.08*
Placental diameter (cm)	0.82 ± 0.26	1.64 ± 0.44*
Placental central thickness (cm)	0.23 ± 0.08	0.56 ± 0.12*
Foetal weight (g)	3.9 ± 0.38	2.2 ± 0.49*
Placental to foetal weight ratio	13.9 ± 3.65	5.5 ± 1.24*
Cord Length (cm)	1.44 ± 0.28	1.68 ± 0.34

Placental weight, placental diameter and placental central thickness were significantly increased (*$P < 0.05$) in IR rats compared to control rats. Foetal weight and placental to foetal weight ratio were significantly reduced (*$P < 0.05$) in IR rats compared to controls.

Table 2: Effect of insulin resistance (IR) on placental morphology in rats

Parameters	Control	IR
Glycogen cells per HPF (n)	82.5±6.5	146.5±7.0*
Trophoblast Giant cells per HPF (n)	32.6±5.9	41.4±11.2*
Spongiotrophoblast cells per HPF (n)	45.8± 9.5	48.9±11.6
Decidua Zone (µm)	75.6 ±8.8	78.2 ±10.5
Junctional Zone (µm)	124.6±28.7	214.8 ±43.1*
Labyrinth Zone (µm)	138.5±24.3	122.6±31.3

The junctional zone of the placenta, number of glycogen and trophoblast giant cells were increased (*$P < 0.05$) in IR compared to control rat placentae (HPF: High Power Field)

Effect of insulin resistance (IR) on maternal serum and amniotic fluid QUICKI values in pregnant rats

QUICKI of maternal serum and amniotic fluid of IR and control rats, derived from maternal and amniotic fluid insulin (Io) and glucose (Go) levels as the inverse log

sum of insulin in micro - unit per millilitre and glucose in milligram per decilitre, are shown in Figure 5. QUICKI of pregnant IR rats were significantly ($P < 0.05$) lower (0.116±0.002, 0.057±0.002) compared to control rats (0.223±0.001, 0.090±0.002) respectively.

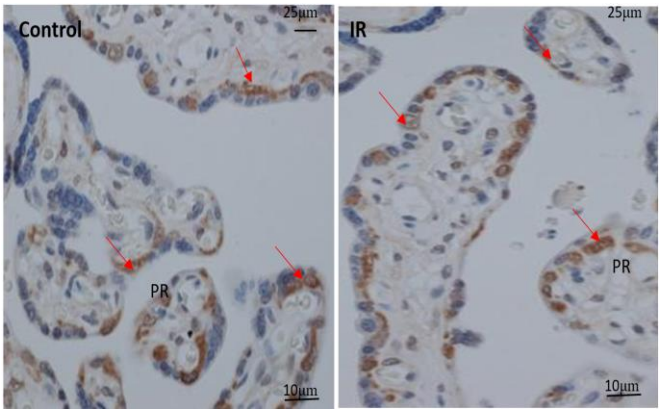

Fig. 9: Photomicrographs of day 19 rat placentae showing immunohistological localization of progesterone receptors (PR- arrows) in control and IR rat placentae. The number of PR per HPF is reduced in IR compared to control rat placantae. Magnification 40x

Effect of insulin resistance (IR) on serum progesterone and oestradiol levels in pregnant rats

Serum progesterone levels were significantly increased ($P < 0.05$) in IR rats (69.6 ± 9.1ng/ml) compared to control rats (33.8 ± 4.6ng/ml). Serum oestradiol levels were however not significantly different in IR rats (16.9 ± 1.54ng/ml) compared to control rats (17.5 ± 1.87ng/ml) as shown in Figure 6. Picture of Day 19 foetuses and placentae from insulin resistant and control pregnant rats is presented as Figure 7 at X40 magnification.

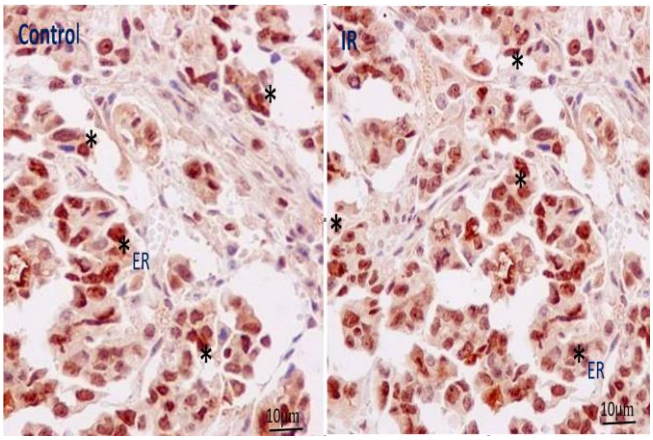

Fig. 10: Photomicrograph of day 19 rat placenta showing immunohistological localization of oestrogen receptors (ER-Asterisks) in control and insulin resistant (IR) rat placentae. 100x. Scale bar -10µm.

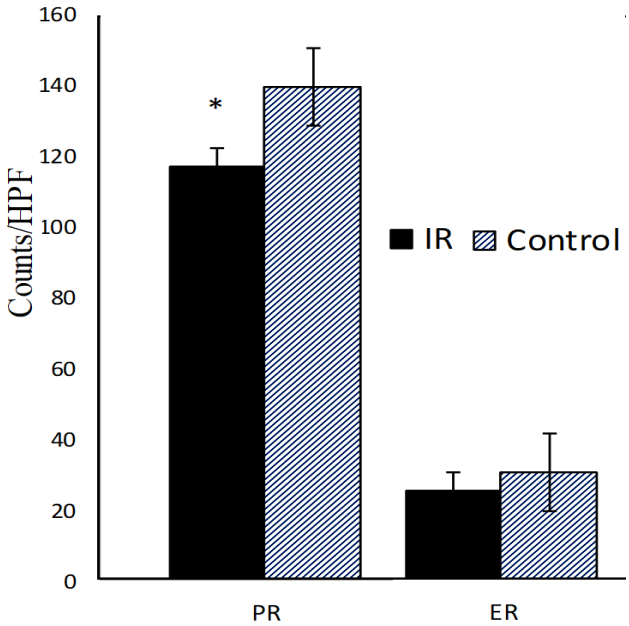

Fig. 11: Progesterone receptor (PR) and oestrogens receptor (ER) counts in control and insulin resistant (IR) rat placentae. PR counts are reduced (*$P < 0.05$) in IR placentae compared to controls. No significant difference was observed in ER counts of IR and control placentae.

Effect of insulin resistance (IR) on placental glycogen-containing cells in rats

The numbers of glycogen-containing cells (GC) in the placentae of IR and control rats are shown in Table 2 and Figure 8. The number of GC per high power field (HPF) in the placentae of IR rats (146.5±7.0 counts/HPF) were significantly increased ($P < 0.05$) compared to control rats (82.5±6.5 counts/HPF).

Effect of insulin resistance (IR) on trophoblast giant cells (TGC) in rat placentae

The numbers of trophoblast giant cells (TGC) in the placentae of IR and control rats are shown in Table 2 and Figure 8. The number of TGC per high power field (HPF) in the placentae of IR rats (41.4±11.2 counts/HPF) were significantly increased ($P < 0.05$) compared to control rats (32.6±5.9 counts/HPF).

Effect of maternal diabetes on placental junctional zone in rats

The size of junctional zones of IR and control rat placentae is shown in Table 2 and Figure 8. The junctional zones of IR rats (214.8±43.1µm) were significantly enlarged ($P < 0.05$) compared to control rats (124.6±28.7µm).

Effect of insulin resistance on progesterone (PR) and oestrogen (ER) receptors expression in pregnant rat placentae

The immunohistological expression of progesterone receptors in control and IR pregnant rat placentae are shown in Figure 9 while that of oestrogen receptors in control and IR pregnant rat placentae are shown in Figure 10. PR and ER counts per high power field (HPF) in control and IR pregnant rat placentae are shown in Figure 11. PR counts per HPF were significantly reduced ($P < 0.05$) in IR (117.0±12.5) compared to control rats (139.5±22.5) while there was no significant difference in ER counts between the two groups.

DISCUSSION

Foetal exposure to a diabetic intra-uterine environment is known to influence the aetiology of metabolic disorders in the offspring later in life, a phenomenon known as foetal programming. However, the underlying mechanisms and the role of pregnancy hormones and the placenta in foetal programming are incompletely understood. The present study therefore used nutritional exposure to high fructose diet to develop an insulin resistance model (Suga *et al.,* 2000; Basciano *et al.,* 2005; Arikawe *et al.,* 2008; Iranloye *et al.,* 2011). This model was used to investigate the effect of insulin resistance on maternal serum progesterone and oestrogens levels, placental morphology, placental expression of progesterone and oestrogen receptors in rats.

Fructose feeding has been used to induce type 2 diabetes mellitus in animal models (Suga *et al.,* 2000; Basciano *et al.,* 2005; Arikawe *et al.,* 2008; Iranloye *et al.,* 2011). In the present study, successful induction of insulin resistance was evidenced by hyperglycaemia and hyperinsulinemia after 12 weeks of chronic fructose feeding. Fasting blood glucose levels increased progressively in the insulin resistance group from the 5th week and diabetic levels (>120mg/dl) were reached at the 12th week. During pregnancy, maternal fasting blood glucose (FBG) level was higher in the insulin resistance group, while the control rats had values below 90mg/dl. Maternal serum insulin and C-peptide levels were over two - folds the control, while the quantitative insulin sensitivity check index (QUICKI) of IR rats were reduced in this study. This is an indication of a reduction in insulin sensitivity in the insulin resistant pregnant rats.

Amniotic fluid glucose level of IR rats was significantly increased ($P < 0.05$) in this study, this suggest an increased influx of glucose from maternal circulation to foetal circulation through the placenta. The transport of glucose through the placental is essential for foetal growth and development, as it serves as the sole foetal energy source (Magnusson-Olsson *et al.,* 2006). Movement of glucose across the placenta is rapid due to the high capacity of the placenta to transport nutrients, with saturation only being reached at a very high glycaemic maternal blood concentration above 350mg/dl (Desoye and Myatt, 2004).

The present study also showed that amniotic fluid insulin was increased in the insulin resistant rats. Maternal insulin however cannot cross the placental barrier due to its high molecular weight (Dickinson *et al.,* 1990; Barbour *et al.,* 2007) implying that amniotic fluid insulin is of foetal origin (foetal pancreatic beta cells). This finding suggests that maternal hyperglycaemia, leading to an increased influx of glucose to foetal circulation through the placenta, results in a compensatory increase in foetal insulin production, as shown by the increase in amniotic fluid insulin levels observed in the IR rats. A similar result was reported by Saito *et al.,* (2010) in streptozotocin-induced diabetic rats.

Foetal pancreas produces insulin to regulate the increased influx of glucose from the maternal circulation, since maternal insulin cannot cross the placenta barrier (Habibulla *et al.,* 2006). Insulin acts by increasing glucose uptake by foetal cells, thus promoting foetal growth (Fowden and Forhead, 2004). The elevated amniotic fluid insulin levels observed in the IR rats is suggestive of foetal hyperinsulinaemia. Foetal insulin is excreted through foetal urine into amniotic fluid, and has been reported to increase as foetal insulin production increases (Illsley, 2000). Increased amniotic fluid insulin has also been associated with increased foetal growth (Tisi *et al.,* 2011; Jansson *et al.,* 2006), macrosomia (Jansen *et al.,* 2003) and foetal morbidity (Buchanan *et al.,* 1995) in previous studies.

Placental hormones are essential for pregnancy maintenance, they function by exerting autocrine and paracrine effects that regulate decidualization, placental development, angiogenesis, endometrial receptivity, embryo implantation, immunotolerance and foetal development (Costa, 2015). In addition, because placental hormones are released into maternal circulation, the profile of their blood levels during pregnancy has been the target of intense research towards finding potential reliable biomarkers to predict and diagnose pregnancy associated complications.

In the present study, maternal serum progesterone levels were increased in the IR rats, suggesting that progesterone may play a role in increasing insulin resistance state of diabetic pregnancies. However, no significant increase was observed in oestradiol levels of IR rats. This hormonal imbalance may impair foetal development as previously reported by Sinzato *et al.,*

2012. Progesterone, a steroid hormone crucial for the maintenance of pregnancy, has been reported to be involved in maternal metabolic changes during pregnancy, promoting hyperphagia, fat storage and insulin resistance (Butte, 2000).

The effect of progesterone is mediated by its receptors - progesterone receptors (PR), which has been identified in the placenta aside the uterus, suggesting that the placenta itself is a target for progesterone action (Tsai and O'Malley, 1994; Shanker and Rao, 1999). Little is currently known about the expression of PR in the placenta of gestational diabetic and type 2 diabetic pregnancies. However, previous studies have reported that progesterone influences pancreatic beta cell function and contributes to insulin resistance state in pregnancy (Polderman *et al.,* 1994; Freeman, 2010).

In the present study, progesterone receptor expression was down regulated in the placenta of IR rats, this may be due to the increase in maternal serum progesterone levels observed in this study. Previous studies have linked changes in the expression of placental receptors to impaired foetal development (Norberg *et al.,* 1998; Jansson *et al.,* 2002). During gestation, the placenta produces progesterone which through its interaction with progesterone receptors exerts its many effects. Specific intracellular progesterone receptors (PR) have been reported to mediate the genomic signalling of progesterone.

Oestrogens have also been reported to be involved in the development of maternal insulin resistance during pregnancy (Gonzalez *et al.,* 2000; Gonzalez *et al.,* 2002; Barros *et al.,* 2009). In this study, however, no significant difference was observed in maternal serum oestradiol levels and placental expression of oestrogen receptors in IR rats. This finding suggests that placental oestrogen receptors is not impaired in this model of diabetic pregnancy.

Placental weight, placental central thickness and placental diameter of IR rats were increased in this study. This finding was paralleled by an increase in the number of placental glycogen-containing cells, trophoblast giant cells and size of the junctional zones in the diabetic placentae. These findings are suggestive of placental hypertrophy, which may represent its adaptive response to the hyperglycaemic maternal environment, as evidenced by the increased number of glycogen-containing cells in the IR placentae. The increase in placental diameter represents an increased area of uterine attachment and placental exchange area. Increase in placental central thickness has been reported to represent an increase in trophoblast angiogenesis and density of blood vessels (Elshennawy, 2016).

These structural adaptive response of the placenta to maternal insulin resistance were explained by previous studies to be a compensatory mechanism caused by changes in glucose metabolism and hormone production by the placenta (Vitoratos *et al.,* 2010; Martino *et al.,* 2016). A similar observation was reported by Evers *et al.,* 2003, in the placenta of Type 1 diabetic women, and in the placenta of gestational diabetic mothers (Sharmila *et al.,* 2017). Pathak *et al.,* 2010's report was however contrary to this finding, they observed no correlation between streptozotocin (STZ)- induced maternal diabetes and changes in placental morphology in rats. This difference in finding may be due to the model of maternal diabetes used for the study, as STZ induces type 1 diabetes mellitus, via cytotoxic destruction of pancreatic beta cells. The present study however induced insulin resistant state in rats with intact pancreatic beta cells.

Placental to foetal weight ratio was significantly reduced in the IR rats compared to Control rats. This may be due to consequential increase in placental parameters/morphology in the IR rats compared to the Control rats as presented in tables 1 and 2. Yessofou and Moutairou (2011) also reported a similar finding in their study. Placental to foetal weight ratio are predictors of long-term adverse outcome for the offspring as reported by Godfrey in 2002. Decrease in placental to foetal weight ratio may represent an adaptive response by the foeto- placental unit to an unfavourable maternal environment. Findings from this study therefore suggests that progesterone and the placenta play roles in foetal programming of type 2 diabetic pregnancies.

CONCLUSION

The study shows that maternal insulin resistance increases progesterone production, leading to a down regulation of progesterone receptor expression in the placenta. The placenta also adapts structurally to maternal insulin resistance, a form of type 2 diabetes mellitus, by increases in weight, central thickness, diameter and trophoblast giant cells.

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