جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة بابل/ كلية العلوم
قسم علوم الحياة

تأثيرات اليود على الغدة الدرقية والدماغ لأمهات وأجنة الجرذان البيض

أطروحة مقدمة إلى

مجلس كلية العلوم – جامعة بابل وهي جزء من متطلبات نيل شهادة

الدكتوراه فلسفة في العلوم/علوم الحياة / علم الحيوان.

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بكالوريوس علوم الحياة/ جامعة المثنى (٢٠١٢)
ماجستير علوم حياة - فسلجة حيوان / جامعة المثنى (٢٠١٥)

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Republic of Iraq
Ministry of Higher Education
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University of Babylon
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Effects of Iodine on Maternal and Embryonic Thyroid and Brain in Albino Rats

A Thesis

submitted to the Council of College of Science,University of Babylon, in partial fulfillment of the Requirements for the Degree of Doctor of philosophy in Science of Biology / Zoology.

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الخلاصة

اليود عنصر مهم جداً للجسم خاصة انه يدخل في تكوين هرمونات الغدة الدرقية التي تمثل اساس الايض الغذائي للجسم وتوليد الطاقة والمحافظة على حرارة الجسم اضافة الى تدخلها في طبيعة النمو كذلك التطور والتكوين للدماغلذا يعتبر اليود سلاح ذو حدين من حيث النقص او الزيادة وبهذه الدراسة ركزنا على دراسة التأثير الفسيولوجي والنسيجي لتراكيز اليود العالية على الغدة الدرقية وادمغة امهات واجنة الجرذان البيض حيث تم قياس هرمونات الغدة الدرقية (الثايروكسين T4 وثلاثي يودوثيرونين T3، الهرمون المحفز للغدة الدرقية TSH) للام ، عوامل نسخ الغدة الدرقية مثل (FOX1، NK2.1. و PAX8)،عوامل النقل العصبي للدماغ (BDNF, Ach) كما تم دراسة التأثير على وزن الجسم وقابلية التكاثر وكذلك تمت دراسة تأثير الزيادة على الطبيعة النسيجية للدماغ والغدة الدرقية لمناقشة وتفسير التغيرات الحاصلة .

اجريت الدراسة في البيت الحيواني ومختبرات كلية الطب جامعة المثنى للفترة الممتدة من شهر نيسان لعام ٢٠٢١ لغاية شهر كانون الاول لعام٢٠٢٢ وقد اشتملت هذه الدراسة الحالية على ٥ مجاميع كل مجموعة تضمنت ١٥ انثى بالغة من الجرذان البيض مقسمة على النحو التالي المجموعة (١): عولجت بجرعة منخفضة من اليود (٠,٠٦ ملغم) ، المجموعة (٢): عولجت بجرعة متوسطة من اليود (١,٨ ملغم)، المجموعة (٣): عولجت بجرعة عالية من اليود (٥ ملغم)، المجموعة (٤): عولجت بجرعة عالية جدًا من اليود (٢٠ ملغم) والمجموعة الأخيرة (٥): وعملت كمجموعة سيطرة (تحكم) .

حيث تم معاملة مجاميع المعالجة باعطائها محلول اليود المخفف فمويا لمدة شهرين ثم تم اضافة الذكور لكافة المجاميع لاجل التزاوج وبعد حدوث الحمل في يوم (١٨-٢٠) من الحمل تم سحب دم من الام لاجل قياس هرمونات الغدة الدرقية ثم قتل الام واخراج الاجنة وتشريحهم لاجل اخذ الغدة الدرقية والدماغ ووزن كل منهم سواء للام او الجنين وتقسيمهم الى قسمين حيث تم حفظ احد الاقسام بالفورمالين لاجل عمل مقاطع نسيجية وفحصها مجهري ومقارنة مجموعة السيطرة مع مجاميع المعالجة ورؤية التغيرات الحاصلة للتراكيب النسيجية اما القسم الثاني فتم استخدامة لاجل عمل السحق النسيجي لاجل قياس العوامل النسخية الدرقية والعصبية .

وقد أظهرت النتائج ان هرمونات الغدة الدرقية للأم (الثايروكسين وثلاثي يودوثيرونين) ارتفعت مع ارتفاع جرعة اليود المعطاة بيما الهرمون المحفز للدرقية انخفض بالمقارنة مع مجموعة السيطرة اماعوامل نسخ الغدة الدرقية للاجنة سجلت انخفاض واضح في القيم مع الزيادة لليود وعكسه سجل في الامهات حيث ارتفعت عوامل النسخ الدرقية مع زيادة الجرعات ’في حين أظهرت عوامل النقل العصبية لدماغ الجنين والام ارتفاع واضح مع ارتفاع الجرعات وبفروق معنويه واضحة مقارنة بمجموعات السيطرة.

الخلاصة

الفحص النسيجي للغدة الدرقية على مستوى الام اظهر حصول تغير بحجم الحويصلات وتضخمها ووجود الخلايا الطلائية عمودية لفرط نشاط الغدة الدرقية وكذلك فرط انتاج المادة الغروي تناسبا مع زيادة كميات اليود المعطاة في حين لوحظ حصول ضمور بحجم الغدة كذلك تغير موقعها تشريحيا حيث اصبح موقعها للاسفل اكثر مقارنة مع الاجنة الطبيعيين اضافة الى صغر حجم الحويصلات الغدية المتكونه وعدم فعاليتها وخمولها وعدم امكانيتها على تكوين المادة الغرية مايوضح اصابة الجنين بخمول الغدة الدرقية بسبب فرط نشاط الغدة الدرقية للام بفعل تاثير زيادة اليود

اما بالنسبة للفحص النسيجي للدماغ اظهر حصول تغيرات طفيفة نوعيا للام تمثل بزيادة الاوعية الدموية وتكاثفها مع زيادة نسبة الجرعة وظهور فجوات تكاد لاتكون واضحة في التغيرات النسيجية على مستوى الجنين تكون واضحة الضرر مع زيادة نسبة الجرعات بدأت من تواجد اوعية دموية وفجوات وزادت لحصول تلف واضح في التكوين وظهور الخلايا العصبية المساندة (الدبقية) في تكوين الدماغ عوضا عن الخلية العصبية الاساسية كذلك ملاحظة تورم وتضخم وتغيرات خلوية بسبب تلف وتاثر الخلايا العصبية بكميات اليود وتاثير هرمونات فعالية الغدة الدرقية بشكل مباشر علي تكوين وتطور الانسجة العصبية كذلك نقص عوامل النقل العصبية كان لها تاثير سلبي في التكوين والتطور النسيجي لدماغ الاجنة وبذلك تم الاثبات بهذه التجربة وحسب النتائج التي تم الحصول عليها ان زيادة اليود الحاصلة للام اثناء الحمل والتي قد تأتي من علاج الغدة الدرقية او المكملات الغذائية او الاكثار من الاطعمة الغنية باليود خاصة توثر على التكوين النسيجي والمعايير الفسلجية للاداء الوظيفي للغدة الدرقية والدماغ على مستوى الام والجنين بصورة عامة وعلى مستوى تكوين وتطور دماغ الجنين بصورة خاصة .

Summary

Iodine is a very important element for the body, especially as it enters into the formation of thyroid hormones, which represent the basis of the body's nutritional metabolism, energy generation, and maintaining body temperature, in addition to its interference in the nature of growth as well as the development and formation of the brain. Therefore, iodine is a double-edged sword in terms of deficiency or excess. In this study, we focused on studying The physiological and histological effect of high iodine concentrations on the thyroid gland and the brains of mothers and fetuses of albino rats, where thyroid hormones (thyroxine T4 and triiodothyronine T3, thyroid-stimulating hormone TSH) for the mother, thyroid transcription factors such as (FOX1, NK2.1, and PAX8) were measured. Brain neurotransmission factors (BDNF, Ach) The effect on body weight and the ability to reproduce was also studied, as well as the effect of the increase on the histological nature of the brain and thyroid gland was studied to discuss and interpret the changes that occurred.

This current experiment - control study was conducted between April 2021-January 2022 and it was carried out at the Animal House and laborites of the Medicine College at the AL-Muthanna University, included 5 groups, each group included 15 adult female white rats, divided as follows: Group 1: treated with a low dose of iodine (0.06 mg), Group (2): treated with a medium dose of iodine (1.8 mg). , Group (3): treated with a high dose of iodine (5 mg), group (4): treated with a very high dose of iodine (20 mg), and the last group (5): served as a control group (control).

Where the treatment groups were treated by giving them solution of diluted iodine orally for a period of two months, after that the males were added to all the groups for mating, and after pregnancy occurred on the day (18-20) of pregnancy, collected blood from only the mother's for measure thyroid hormones, then the mothers were killed with the embryos and take the thyroid gland and brain.

The results showed that the mother's thyroid hormones (T4 and T3) increased with the increase in the dose of iodine given, while the TSH decreased compared to the control group. The transcription factors of the thyroid gland of the fetuses recorded a clear decrease in values with the increase of iodine, and its opposite was recorded in the mothers, where the thyroid transcription factors increased. With increasing doses, while neurotransmission factors to the brain of the fetus and mother showed a clear increase with higher doses and clear significant differences compared to the control groups.

The histological examination of the thyroid gland at the mother, show that there was a change in the size of the vesicles, their enlargement, and the presence of vertical epithelial cells due to hyperactivity of the thyroid gland, as well as an overproduction of colloidal substance in proportion to the increase in the amounts of iodine given, while it was noted that atrophy occurred in the size of the gland, as well as its anatomical location changed, as it became located downward. More compared to normal fetuses, in addition to the small size of the glandular vesicles formed, their ineffectiveness, their lethargy, and their inability to form the slimy substance, which indicates that the fetus has hypothyroidism due to the hyperactivity of the mother’s thyroid gland due to the effect of increased iodine.

When the histological examination of the brain, it showed that there were slight changes qualitatively for the mother, represented by an increase in blood vessels and their thickening with an increase in the dose ratio, and the emergence of gaps that are almost not clear in the histological changes at the level of the fetus, which are clear damage, and the supporting neurons (neuroglia) in the formation of the brain instead of the main neuron, as well as observation of swelling, enlargement and cellular changes due to damage and influence of neurons with iodine amounts and the effect of thyroid activity hormones directly on the formation and development of nervous tissue, as well as the lack of

neurotransmitter factors had a negative impact on formation And the histological development of the brain of the embryos, and thus it was proven in this experiment and according to the results obtained that the increase in iodine that occurs to the mother during pregnancy, which may come from thyroid treatment, nutritional supplements, or an abundance of foods rich in iodine in particular affects the histological composition and physiological criteria of the functional performance of the thyroid gland and the brain At the level of the mother and fetus in general, and at the level of formation and development of the fetal brain in particular.

Dedication

To myself hardworking, ambitious and determined to reach …

To my family who help and supported me along the time …

To everyone who prayed for my success …

Acknowledgements

Praise be to "ALLAH" and to his prophet "Muhammad", my praise and thanks are due for their blessings without which this research would not have been achieved.

I wish to express my deep appreciation to my supervisor Prof. Dr. Ehab Razak Al-Samawy and Prof. Dr. Alaa Tariq Shakir.

Thank and great appreciation to all my family for their help, support, and encouragement especially my sister Huda .

My thanks for my many friends especially(Hassanain, Saja, Hieam, Wassan, Hawraa, Huda).

Certificate

We certify, that this thesis entitled " **Effect of iodine on maternal and embryonic thyroid and brain in albino rats** " and examined the student **"Ghina Hamoudi Hussein Alzubaidi ",** under our supervision in Department of Biology, Faculty of Science – Babylon University, as a part of the fulfillments for the degree of Doctor in philosophy of Science in Biology / Zoology.

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Recommendation of the Head of Department of Biology

Reference to the above recommendations submitted by the supervisors to transmit this thesis to the Commission for Consideration and debate Statement of Opinion.

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Date: / / 2023

Certification of Examining Committee

We, the examination committee, certify that we have read the thesis entitled (**Effect of iodine on maternal and embryonic thyroid and brain in albino rats)** and have examined the student (**Ghina Hamoudi Hussein Alzubaidi**) in its contents, and that in our opinion it is accepted as a thesis for Degree of Doctorate of Philosophy in Science of Biology – Zoology with excellent estimation.

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Dedication

I owe everyone who lent me a hand great thanks especially

To……..my pride and honor, My father

To……the symbol of tender and fountain of compassion who continuously encouraged, who lights my way, My mother

To.....those who my help at life and supported me

along the time, My sisters and brothers

To everyone who prayed for my success

To all researchers who are working

to improve the quality of life

To all of them I dedicate this work

1.1 Introduction

Iodine is a microelement that can be found as a dietary supplement, added to some foods, and naturally in others. Before being absorbed by the gastrointestinal tract, iodine from the meal is transformed into the iodide ion (Patience,2018). Iodide enters the blood, is concentrated by the thyroid gland in the precise levels needed for the manufacture of thyroid hormone, and the majority of the residual quantity is eliminated in the urine (Vought London,1967).

Iodine needs can be estimated using median urine iodine concentrations of 100 g/L, which correspond to daily iodine intake of about 150 ℳg /Ml (Russell *et al*., ,2001). The median urine iodine concentrations in children, adults, pregnant women, and nursing women range from 100 to 199 g/L, 150 to 249 g/L, and >100 g/L, respectively, demonstrating appropriate iodine consumption, Urinary iodine levels below 20 g/L are considered severe iodine insufficiency, though values below 100 g/L in children and non-pregnant adults signal inadequate iodine ingestion (WHO,2007).

Seawater contains iodide ions at a concentration of around 50 g/L, and when these ions are oxidized, elemental iodine is produced, which is very volatile and evaporative. Iodine is then restored to the soil in rain after precipitation. This cycle is insufficient in some areas, causing iodine shortage in the soil, which may also affect crops planted there (Thiry *et al*.,2022). Many nations have tried to address this issue by artificially restoring iodine into the food chain through salt iodization programs since populations in these areas are susceptible to developing an iodine deficiency (Farebrother *et al*.,2015) 90% of the time, the stomach and duodenum are where iodide ions are absorbed. After entering the bloodstream, iodide is carried into the thyroid gland by a sodium/iodide symporter (Ishii *et al*.,2019).

Thyroid hormone is necessary for children's optimal brain development, and the crucial time is during the first three years of life, The most important and curable factor known to contribute to children's brain neurodevelopmental problems is iodine deficiency, In order to better understand and prevent iodine deficiency illnesses, research has long focused on the connection between iodine nutrition, thyroid function in pregnant women, and brain development in offspring (QIAN, 2020).

The discovery of the thyroid transcription factors (TTFs): Homo box protein (NKX2-1), Forkhead box protein (FOXE-1), and Paired box gene (PAX-8) have been crucial for understanding thyroid differential and development, congenital thyroid disorders, thyroid cancer and There TTFs are required for maintaining the adult thyroid's functional , The discovery of the genes driving human congenital thyroid abnormalities and thyroid cancer has been made easier by a better understanding of the mechanisms underpinning thyroid organogenesis and differentiation, Polymorphisms, epigenetic modifications, and mutations in the genes that encode the TTFs have all been connected to thyroid disorders (Fernandez *et al*.,2015).

Acetylcholine (Ach) is a neurochemical (neurotransmitter) more precisely, as chemical messenger that allows connect between neurons and other specialized cells like myocytes and cells in glandular tissues (Waxenbaum *et al*.,2021).

Brain-derived neurotrophic factor (BDNF) also controls neurotransmitter in CNS synapses and regular the neuronal growth and differential (Castrén and Antila (2017) and Wang *et al.* (2022)). There are three compartments of neuromuscular junctions are axon terminals, per synaptic Schwann cells, and muscler fibers that contain it’s and receptors (Garcia *et al*., 2010b).

1.2 The aims of this study

1- Correlate the relationship between mother's iodide supplement during pregnancy and effect on embryonic thyroid gland development .
2- Correlate the relationship between mother's iodides excesses during pregnancy and effect on embryonic brain and neurotransmitters faction .
3- Measurement effect of iodine on thyroid hormones(T3,T4 and TSH), thyroid transcription factors(NKX2-1,FOXE-1 and PAX8) .
4- Measurement effect of iodine on brain factors(Ach and BDNF) .
5- Study the histological changes for thyroid and brain tissue in mother and embryo .

2. Literature Review.

2.1 Iodine.

Thyroid hormones need iodine as a key ingredient and is necessary for appropriate metabolism and cognitive function throughout life (Velasco *et al*.,2018) . Iodine and thyroid hormones from the mother are necessary for fetal neurogenesis throughout pregnancy, which includes axon and dendritic development, synapse formation, myelination, and neural migration, in the United Kingdom (UK), dairy products and seafood are the main dietary sources of iodine Supplements are also available (Bouga *et al*.,2018) .

Currently, there is no requirement for iodine fortification in the UK, and there are only a small number of retail locations that sell iodized salt (Bath *et al*., 2014). Additionally, the National Institute for Health and Care Excellence's (NICE) antenatal care guidelines don't mention iodine nutrition (National Institute for Health and Care Excellence ,2019).

Children fulfilled the World Health Organization's (WHO) criteria of iodine sufficiency, according to the most recent National Diet and Nutrition Survey, whereas women of childbearing age (16-49 years old) did not (Public Health England ,2016) . Pregnancy increases the body's need for iodine by around 50%, making this group potentially susceptible to iodine shortage (Velasco *et al*.,2018).

This may be made worse by avoiding iodine-rich foods because you follow a vegetarian or vegan diet, by forgoing foods like smoked fish, raw shellfish, marlin, and shark while pregnant, by being told to limit intake of oily fish to two servings per week due to worries about heavy metal toxins, and by having trouble managing your nausea and vomiting symptoms (National Health Service ,2020) .

The most common cause of mental impairment in children globally is iodine shortage in pregnancy (Aburto *et al*.,2014). It has been indicated that modest iodine deficit in pregnancy may lead to lower intelligence quotient (IQ) scores in children as well as an increased risk of perinatal problems, while the consequences of mild to moderate iodine deficiency are less well documented (Verhagen *et al*., 2020 ; Abel *et al.*,2020). This has been confirmed by certain population studies, which have shown conflicting links between maternal iodine deficiency-induced thyroid dysfunction and the cognitive and behavioral functioning of the children (Wang *et al*.,2018).

2.2Source of Iodine.

2.2.1 Food and Food Products.

Despite the fact that the concentration varies and most foods have low native iodine levels, diets from the sea, especially those including specific seaweeds, are high in iodine (Nagataki ,2008) .Consuming seaweed is fundamental to various cultures, such as those in Japan, Korea, and other parts of East Asia the majority of the population in these areas tolerates daily doses of iodine at mg dosages well however, school-age children, adults, preterm neonates (Farebrother *et al*.,2019).

The breastfed newborns have all been documented to experience thyroid tissues brought on by high iodine intakes from seaweed (Fuse, 2017).

Many diets rely heavily on milk products for their iodine content, which might vary depending on the dairy industry (van der Reijden *et al*.,2017). Iodized cattle feed and minerals supplements are frequently given to dairy cows in excess of what they need to eat (Van der Reijden *et al*.,2019).

Other milks, such as camel and goat milk, reportedly have high concentrations iodine and to be significant dietary contributors in some areas ,Since it includes significant levels of iodine, the food color erythrosine is no longer commonly utilized in the USA (Pichel and Vivar. 2017).

2.2.2 Breast Milk and Infant Compound.

Avital micronutrient throughout the first 1000 days of life is iodine (Farebrother *et al*.,2019) . Depending on the mother's iodine consumption, the amount of iodine in breast milk varies greatly. In iodine-sufficient communities, iodine is preferentially partitioned into breast milk to protect baby needs if consumption is borderline (Dold *et al*., 2017).

In regions with excessive iodine intakes, a high breast milk iodine content (BMIC) has been documented (Aakre *et al*.,2016) . Uncertainty persists on whether iodine secretion in to the breast milk is actively down regulated at high maternal iodine intakes, with very few exceptions, infant formula milk must contain iodine to mirror the make up of breast milk, Infant formula (compound) in the United States has 5–75 g of sugar per 100 calories, but EU rules specify a stricter range of 15–29 g of sugar per 100 calories (The European Union, 2015) .

2.2.3 Water.

Concentrations are largely related to the well's depth, the kind of bedrock it was filtered through, and its closeness to a marine environment (Johnson,.2003; Yang *et al*.,2021). Iodine-rich water that has been involved with thyroid dysfunction and goiter in some places (Claim Cui *et al.*, 2017). And may serve as the primary sources of dietary iodine for some groups (Jwan Jawzali, 2017).

2.2.4 Dietary Supplements.

Iodine-containing prenatal supplements are advised for women with insufficient food intake during pregnancy and breastfeeding, but if high maternal consumption, the risk of fetal or neonatal issues increases (WHO, 2007). In the European Union, dietary supplements are subject to the same regulations as foods, and an uniform legal framework governs the ingredients and sources of dietary supplements (Lee *et al.*,2017 ; European Food Safety Authority, 2018).

The Food and Drug Administration in the US has been given the power to create rules for the production of dietary supplements as well as for health claims and labelling , there are disparities in the way dietary supplements are regulated (Consumer Healthcare Products Association, 2018) . According to an Italian study, more than half of the dietary supplement was examined had iodine levels that were higher than what was considered reasonable (Restani *et al*., 2008) .

A study of prenatal multi vitamins sold in the USA discovered considerable discrepancies between label information's and laboratory assessment; 25 brands that contained kelp had daily doses ranging from 33 to 610 mg, the latter amount being over three times the 220 mg daily dietary recommendation (Leung *et al*.,2009).

It is recommended to utilize potassium iodide in vitamin preparations rather than kelp supplements for this reason, which may be explained by fluctuations in the natural iodine content of kelp (Leung and Braverman ,2014).

2.3 Iodine Metabolism.

Iodide (I), iodate (IO3), which is generally utilized to iodize salt, and bound iodine are the three main types of iodine that are eaten, more than 90% of ingested iodide was absorbed into the duodenum (Dela Vieja *et al*.,2018). Organically bound iodine is digested and the released iodide will be absorbed, whereas iodate is transformed to iodide in the gut before being absorbed, the sodium/iodide symporter (NIS), which is present on the apical plasma membranes of enterocytes in the jejunum, duodenum and ileum, mediates this conversion (Ravera *et a*l.,2017).

In addition, it's thought that the sodium multivitamin transporter (SMVT) and the cystic fibrosis transmembrane conductance regulator (CFTR) have a role in iodide absorption in gut, Iodide enters the circulation after being absorbed by molecular processes that are still not fully understood (Ravera *et al*.,2017). Both intestinal absorption and the deiodination of T4 and T3 by deiodinases in peripheral tissues affect the blood's concentration of iodide (Bianco and Conceiço, 2018).

Through action of NIS, which is present in the basolateral plasma membrane of the thyrocytes, circulating iodide is either eliminated in the urine or taken up by the thyroid gland, immunohistochemistry was first used to identify NIS expression in the kidney, namely in the basolateral membrane of distal tubular cells, indicating that these cells are involved in excreting iodide (Spitzweg *et al.*,2001). Later studies, however, revealed NIS expression on the apical membrane of cells from the proximal then the cortical collecting tubes (Ravera *et al*.,2018).

As a result, these two conflicting processes may cause iodine to be excreted through the urine, Although the thyroid gland only retains around 10% of the iodine that enters the body through the digestive system, it can accumulate more than 80% of the iodide in the circulation in conditions of chronic iodine shortage (Iqbal *et al*.,2021).

Both the fetal thyroid and the mammary gland clear circulating iodide during gestation and lactation, with the fetal thyroid starting to function in the middle of the second trimester of pregnancy and the mammary gland concentrating iodide in milk during breastfeeding to ensure proper thyroid functioning in the infant (Sterrett,2019).

To ensure the mother's transmission of iodine to the new fetus, the placenta expresses PENDRIN, a sodium-independent chloride-iodide exchanger and NIS, the finding that the placental production of NIS at 8–10 weeks is already significant of gestation, when the fetal thyroid is not yet able to synthesize thyroid hormones , suggests that at this time, iodide may have further physiological uses such as defense against infections , antioxidant activity (Chan *et al*.,2009).

The iodide concentration is guaranteed by NIS expression in the basolateral membrane of mammary alveolar cells in the mammary gland, then secretion into the milk is carried out by the iodide transporters CFTR, ANO1, and PENDRIN, which are found on the apical side of the cell membrane (Tamma and Dossena, 2022).

2.4 Iodine Important and Correlation with Pregnancy.

The quantity of iodine that our bodies require depends on a variety of conditions, including physiological changes like pregnancy, the need for iodine rises dramatically during pregnancy due to three factors: (i) I a 50% increase in maternal thyroxine (T4) production required to maintain maternal euthyroidism and meet the fetus's thyroid hormone needs; (ii) the need to transfer iodine to the fetus for fetal production of thyroid hormone, particularly in later gestational stages; and (iii) a likely increase in renal to ensure good fetal neurodevelopment throughout pregnancy, enough iodine consumption is necessary for both the mother's and the fetus' generation of thyroid hormones (Glinoer , 2004).

Numerous negative health effects are brought on by eating inadequate amounts of iodine. According to (WHO/UNICEF/ICCIDD, 2007). The effects of severe iodine deficiency (ID) during pregnancy include spontaneous miscarriage, stillbirth, congenital malformations, and endemic cretinism (Toloza *et al*.,2020).

The recommended nutritional intake (RNI) for iodine during pregnancy was recently increased by WHO/UNICEF from 200 to 250 g/d. urinary iodine concentration (UIC) and thyroid volume (goiter rate) (GR) can be used to measure inadequate iodine intake in children and women (Zimmermann , 2008). Urinary iodine concentration testing is advised for pregnant women to determine their iodine levels (UIC), UIC is a great predictor of recent iodine consumption since > 90% of dietary iodine eventually shows up in the urine (Maria J. Gunnarsdottir *et al* ., 2012). To address iodine deficiency in nations with salt iodization programs, the World Health Organization advises that household iodized salt usage should be at least 90%. Iodized salt coverage at the household level has considerably grown in Ethiopia over time, going from 15% in 2011 to 89% in 2016 (CSA and ICF, 2016).

2.5 Thyroid Gland.

It's common to compare the thyroid gland two lobes to a butterfly or a bow tie, It is located in the neck, halfway between the C5 and T1 vertebrae, immediately behind the thyroid cartilage (Adam's apple) of the larynx, it comprises two principal lobes (the right and left), which are connected by an isthmus, a more compact piece of tissue (Dorion ,2017) .

The pyramidal lobe, which is generally triangular in shape, is a third lobe that is present in around half of people, the adult thyroid gland has four principal lobes, each measuring about 2 cm in width and weighing between 15 and 30 g ram, four small parathyroid glands, which operate separately from the thyroid, are embedded in the back of the thyroid (Benvenga ,2018).

2.5.1 Thyroid Gland Histology.

A thin, tight covering of connective tissue called the thyroid capsule surrounds the thyroid gland and transmits septa into the thyroid parenchyma, separating it into numerous lobules, twenty to forty circular to oval follicles, each approximately 30 to 500 microns in diameter, are present in each thyroid lobule (Skandalakis, 2004).

The thyroid gland in mature males has around 106 thyroid follicles, the cuboidal epithelial cells that surround each thyroid follicle have a core store of colloid, which they release when thyroid stimulating hormone (TSH) is present (Williams and Bannister, 1995).

The thyroid follicles are divided by a thin layer of connective tissue called the stroma , which has lymphatic, blood, and nerve vessels, the thyroid follicle lumen contains colloid, which is scalloped and pale in thyroid follicles with active secretory activity and more flocculent ("like a clump or tuft of wool") and basophilic in older adults (Skandalakis, 2004).

The parafollicular or C cells, which are descended from the neural crest and comprise the second group of thyroid secretory cells, contain and produce the calcitonin hormone , in the inter-follicular stroma (in the upper poles of the thyroid lobes), they can be found as single cells or clumping together in small clusters (Skandalakis, 2004).

A stain for calcitonin is used to identify the parafollicular or C cells, which make up about 0.1 percent of the thyroid gland and are found in ten parafollicular cells per low power field in adults, the C cells have oval nuclei and pale to clear cytoplasm , making it difficult to distinguish them with histamine and eosin stain (H&E) (Das *et al*.,2017).

The thyroid gland has two lateral lobes with superior and inferior poles joined by a median isthmus, with an average height of 12 to 15 mm, overlaying the second to fourth tracheal rings, it's shape can range from a H to a U shape, the gland may have two separate lobes instead of the isthmus, the superior poles of the lateral thyroid lobes diverge laterally at the level of the oblique lines on the thyroid cartilage lamina , with each lobe measuring an average of 50 to 60 mm (8 to 10 ml in volume) (Williams and Bannister, 1995).

At the level of the fifth tracheal cartilage, the lower poles divide laterally, the thyroid gland weight fluctuates, it typically ranges from 15 to 30 g in adults , the thyroid gland is somewhat larger in women, and it enlarges during menstruation and pregnancy (Alessandro M Vannucchi *et al*., 2017).

About 50% of people have a pyramidal lobe, which frequently ascends from the isthmus or the neighboring portion of either thyroid lobe (more frequently the left), the hyoid bone, to which it may be connected by a fibrous or fibromuscular band, the levator of the thyroid gland (Williams and Bannister, 1995).

A loosely connected fascia that forms from the deep cervical fascia's split into anterior and posterior divisions envelops the thyroid gland, the visceral fascia, also known as the genuine thyroid capsule, is a thin, tightly adherent layer of fibrous tissue that releases septa that intrude into the gland to generate pseudo-lobules, the visceral fascia, a division of the middle layer of the deep cervical fascia, connects the thyroid gland to the laryngeal bone (Skandalakis, 2004).

The anterior and posterior suspensory ligaments are formed from this tissue's condensed form, the anterior suspensory ligament extends to the cricoid and thyroid cartilage from the superior-medial portion of each thyroid lobe, the first and second tracheal rings, as well as the side of the cricoid cartilage, are joined to the posterior-medial portion of the gland by the posterior suspensory ligament (Berry ligament) due to the thyroid gland tightly linked gland to the laryngeal bone, the thyroid gland and related components move when swallowing (Fancy *et al*.,2010).

٢,٥,٢ Thyroid Hormones.

The two major active hormones secreted by the thyroid gland are thyroxine, and triiodothyronine, commonly called T4 and T3, respectively, both of these hormones profoundly increase the metabolic rate of the body; the thyroid gland a butterfly-shaped endocrine gland located in the neck, is the primary organ in this endocrine system, the thyroid gland is one of the largest of the endocrine glands, normally weighting 15 to 20 grams in adults, the thyroid gland is composed of a large numbers of closed follicles (100 to 300 micrometers in diameter) (Arthur,2006).

The major constituent of colloid is the large glycoprotein thyroglobulin, which contains the thyroid hormones within its molecule; however almost all the thyroxine is eventually converted to triiodothyronine in the tissues, so that both are functionally important (Burtis, 2006).

Thyroid secretion is controlled primarily by thyroid-stimulating hormone (TSH) secreted by the anterior pituitary gland (Thomas, 2005) . The basal membrane of the thyroid cell has the specific ability to pump the iodide actively to the interior of the cell, this is called iodide trapping, the thyroid cells are typical protein-secreting glandular cells (Dennis *et al.*, 2005; Arthur, 2006) .

These thyroid hormones T4 and T3 are derived from tyrosine and iodine when released into circulation, they ultimately produce a diverse systemic organic and metabolic response , iodine binds with amino acid tyrosine in Tg molecule within follicular cells to form monoiodotyrosine (MIT) and di-iodotyrosine (DIT) as (Figure2-1) (Wendy *et al.*, 2007) . The thyroid gland stores thyroid hormones that are released into the body as needed (Sembulingam and Sembulingam,2012) . The human metabolism is significantly influenced by thyroid hormones (Stathatos, 2019) .

(T3) and (T4) are the two vital hormones that the thyroid gland synthesizes in the body, iodine metabolism initiates the thyroid hormones synthesis pathways (Mansourian, 2011). T3 is created to a lesser than T4 in the generation of thyroid hormones, T3 however, is more activity than T4 About 90% of the gland's output is made up of T4 , while the remaining 9% is made up of T3, the third small hormone that the thyroid gland secretes, making up 1% of the total production and not physiologically necessary, is called reverse T3 (rT3) (Danzi and Klein, 2003).

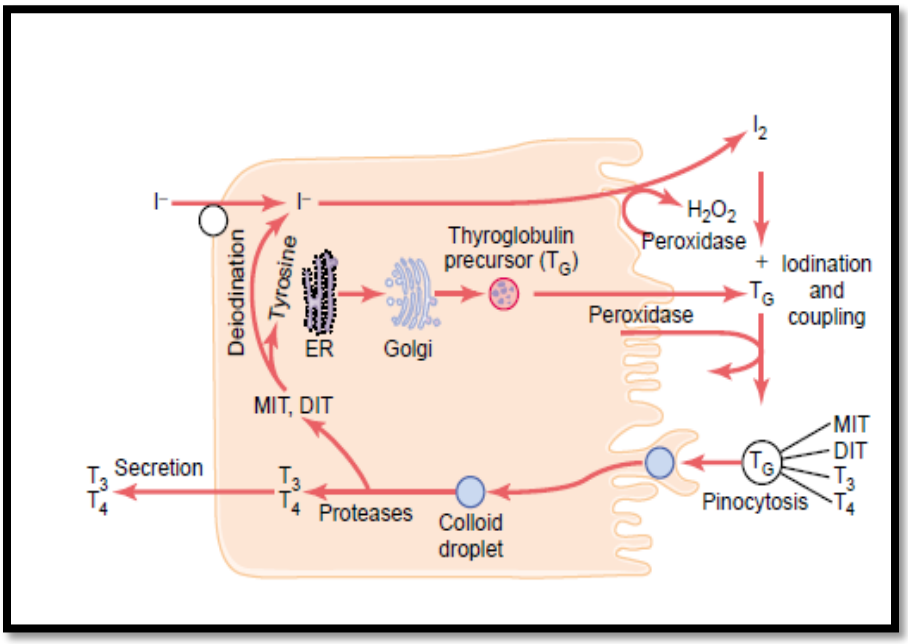


Figure (2-1): Mechanism of Thyroxin and Triiodothyronine Formations in the Target Cell in Thyroid Gland (Arthur, 2006).

Reactions are rapid' stimulated by TSH , T4 is formed by coupling of two molecules of (DIT) and tri-iodothyronine (T3) is formed by coupling of one molecule of MIT with one molecule of DIT (Lawrence *et al.*, 1999; Dennis *et al.*, 2005; Arthur, 2006; Wendy and Jean, 2007 ; Angela *et al.*, 2010; Wang *et al.*, 2011) .

Reaction requires enzyme thyroid peroxidase (TPO) stimulated by TSH, T_3 is the most active form T_4 has only minimal hormonal activity; however T_4 is much longer lasting and can be converted to T_3 (in most tissues) and thus serves as a reservoir for T_3, A3rd form of thyroid hormone, reverse T_3 (rT_3), has no metabolic activity; levels of rT_3 increase in certain diseases; the binding of iodine with the thyroglobulin molecule is called organification of the thyroglobulin (Wang *et al.*, 2011).

oxidized iodine even in the molecular form will bind directly but very slowly with the amino acid tyrosine, in the thyroid cells, however, the oxidized iodine is associated with an iodinase enzyme that causes the process to occur within seconds or minutes (Elio and Ettore, 2004). Therefore, almost as rapidly as the thyroglobulin molecule is released from is secreted through the apical cell membrane into the follicle, iodine binds with about one sixth of the tyrosine amino acids within the thyroglobulin molecule (Arthur, 2006).

The liver has an important role in thyroid hormone metabolism it is the manufacturer of proteins that bind thyroid hormone, such as thyroxine-binding globulin (TBG), pre-albumin and albumin (Miau and Yun, 1995) . Transport and binding of thyroid hormone is carried out by thyroxine- binding globulin (TBG) (Burtis *et al.*, 2006; Jennal, 2006).

Thyroxin-binding globulin TBG carries the major part of both circulating T4 and T3(as well as reverse T3), and therefore quantitative or qualitative changes in TBG concentration have a high impact on total serum T4 and T3 shown in (Figure 2-2) (Ulla and Krogh, 2007).

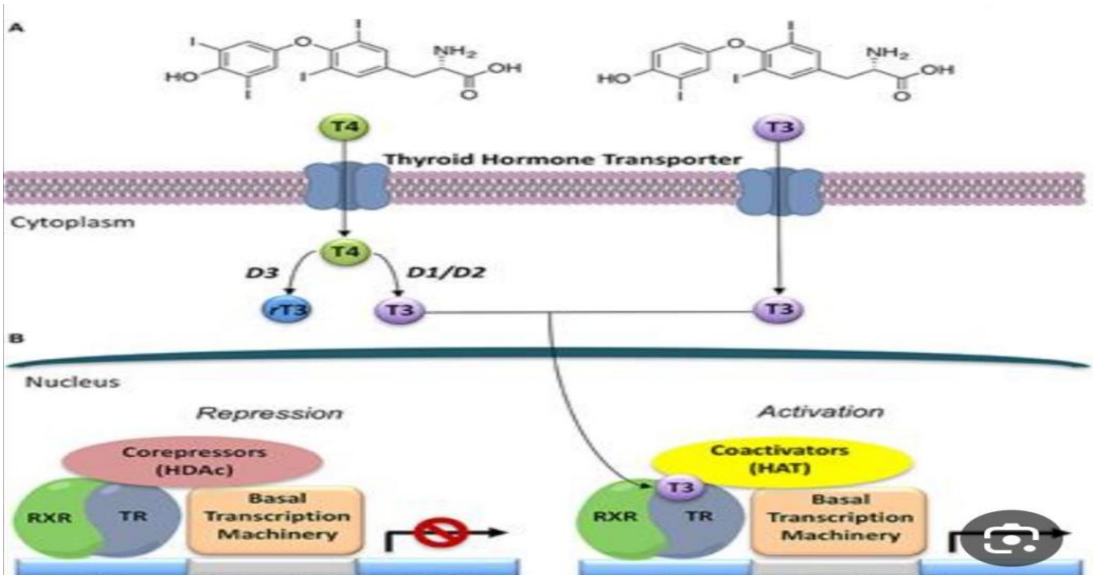


Figure (2-2): Mechanism of Thyroid Hormones Transporter (Rebecca *et al.*, 2011).

2.5.2.1Thyroid-Stimulating Hormone(Thyrotropin) (TSH):

Thyrotropin or TSH is a glycoprotein hormone composed of two polypeptide alpha and beta chains, it is produced in the anterior pituitary, the hormone has a short half-life of minutes to a few hours when compared with other hormones such as steroids, TSH binds to a specific TSH receptor found in the cytoplasmic membrane of thyroid cells ; it releases second messengers that activate cAMP and protein kinase and stimulate further biochemical cascades (Arthur, 2006) .

Thyrotropin is transported free, not bound to a protein carrier in body fluids, it is metabolized to active states from pre- and prohormones when active TSH is needed in part from thyrotropin-releasing hormone (TRH) stimulation , in response to TSH and to other adrenergic neuropeptides binding to cytoplasmic membrane receptors (Dennis *et al.*, 2005; Wendy and Jean,2007) **.**

The relationship between free T4 and TSH (when the pituitary-thyroid axis is intact) is an inverse log-linear relationship; therefore, small changes in free T4 result in large changes in serum TSH concentrations (Rebecca *et al.,* 2011).

2.5.2.2Thyroxin (T4) :

Thyroxin (T4) is produced by the thyroid gland in the body under the control of the pituitary and brain, Triiodothyronine (T3) must be converted from T4 by a type 1 (D1) or type 2 (D2) deiodinase enzyme before it may become active (Larsen *et al*.,2013). The primary component necessary for the synthesis of thyroxine is iodine, Iodide is required from the ingested iodine to generate a typical level of thyroxine (Hall andGuyton,2011) .

2.5.2.3 Triiodothyronine (T3) :

Triiodothyronine (T3) the active thyroid hormone, is produced by the thyroid gland, Most physiological activities in the body, including growth, metabolism, and heart rate, depend on it. When it comes to potency T3 is four times stronger than T4 yet T4 has a longer duration of action T3, T3 has a quicker action than thyroxin because of its lower affinity for plasma proteins and speedy release (T4) (Begam and Rajalakshmi, 2022)

Triiodothyronine's metabolically active form's effects (T3) increases the basal metabolic rate, increases heart rate, cardiac output, and blood pressure. positive influence on catecholamine effects enhances brain function, increases protein catabolism, thickens the endometrium in women (Grais *et al*., 2014 and Bernal *et al*., 2022).

2.5.3 Regulation and Secretion.

Regulation of thyroid hormones starts in the brain, the hypothalamic-hypophyseal portal system transports thyrotropin-releasing hormone (TRH) from the hypothalamus to the anterior pituitary gland, TRH stimulates the thyrotropin cells in the anterior pituitary to create thyroid-stimulating hormone (TSH) (Shahid *et al*.,2022).

The peptide hormone TRH is created by the cell bodies in the periventricular nucleus (PVN) of the hypothalamus, these cell bodies project their neurosecretory neurons into the hypophyseal portal circulation, where TRH may accumulate before reaching the anterior pituitary, the thyroid-releasing hormone receptor (TSH-R) is located on the basolateral side of the thyroid follicular cell and is activated when TSH is released into the circulation (Guyton,2011).

The adenylyl cyclase and intracellular levels of cAMP are activated when the Gs-protein coupled receptor TSH-R is activated, protein kinase -A is made active by the increased cAMP , protein kinase (PKA) changes the functionality of several proteins by phosphorylating them, below are the five stages of thyroid synthesis (Shahid *et al*.,2022).

1. **Synthesis of Thyroglobulin**: Thyrocytes in thyroid follicles create a protein called thyroglobulin (TG), TG is a precursor protein that lacks iodine and is stored in the lumen of follicles, the primitive endoplasmic reticulum produces it, it is packed into vesicles by the Golgi apparatus and then exocytosed to the follicular lumen (Mason and Price,2016).
2. **Iodide uptake**: Kinase of Phosphoprotein Iodide is transported into thyrocytes from the circulation by basolateral Na+-I- symporters, which are activated by Na+-K+-ATPase, more actively as a result of phosphorylation, iodide then diffuses from the basolateral side to the apex of the cell, where it enters the colloid via the pendrin transporter(Shahid *et al*.,2022).

3. **Iodination of thyroglobulin**: Protein kinase -A phosphorylates and activates thyroid peroxidase (TPO), three of TPO's roles are oxidation, organification, and coupling reaction (Shahid *et al*.,2022):

i. Oxidation: TPO uses hydrogen peroxide to convert iodide (I-) in to iodine (I2), hydrogen peroxide is produced by the apical enzyme NADPH-oxidase for TPO(Guillamón-Vivancos *et al*., 2015).

ii. Organization: The thyroglobulin protein's tyrosine residues are linked to I2 by TPO, it generates both monoiodotyrosine (MIT) and diiodotyrosine (DI) (DIT), there are one iodine-containing tyrosine residue at MIT and two iodine-containing tyrosine residues at DIT (Guillamón-Vivancos *et al.,* 2015).

iii. Iodinated tyrosine residues are combined by TPO to produce triiodothyronine (T3) and tetraiodothyronine, when two DIT molecules combine to form T4 from MIT and DIT, T3 is produced (Mason and Price,2016).

4. **Storage**: Coupled with thyroglobulin, thyroid hormones are stored in the follicular lumen. (Shahid *et al*.,2022).

5. **Release**: In the ways outlined below, thyroid hormones are released by thyrocytes into the network of fenestrated capillaries(Guyton,2011):

a. Thyrocytes endocytose iodinated thyroglobulin for uptake (Gereben et al., 2008).

b. Iodinated thyroglobulin-containing lysosomes and endosomes mix (Guyton,2011).

c. MIT, DIT, T3, and T4 are formed from thyroglobulin by endolysosomal proteolytic enzymes(Gereben *et al.,* 2008).

d. T3 (20%) and T4 (80%) can be released into fenestrated capillaries thanks to the MCT8 transporter (Guillamón-Vivancos *et al.,* 2015).

e. Deiodinase enzymes remove iodine molecules from DIT and MIT, it is possible to recover iodine and distribute it to a pool of iodide inside the cells (Mason and Price,2016).

TSH (from the anterior pituitary gland) increases thyroid secretion, TSH also known as thyrotropin, is an anterior pituitary hormone; this hormone increases the secretion of thyroxine and triiodothyronine by the thyroid gland, its specific effects on the thyroid gland, feedback effect of thyroid hormone to decrease anterior pituitary secretion of TSH (Arthur, 2006). Shown in (Figure 2-3); T4, and to a small degree T3 circulating in the serum inhibits secretion of TSH and TRH, thereby completing the feedback cycle (Jennal, 2006) .

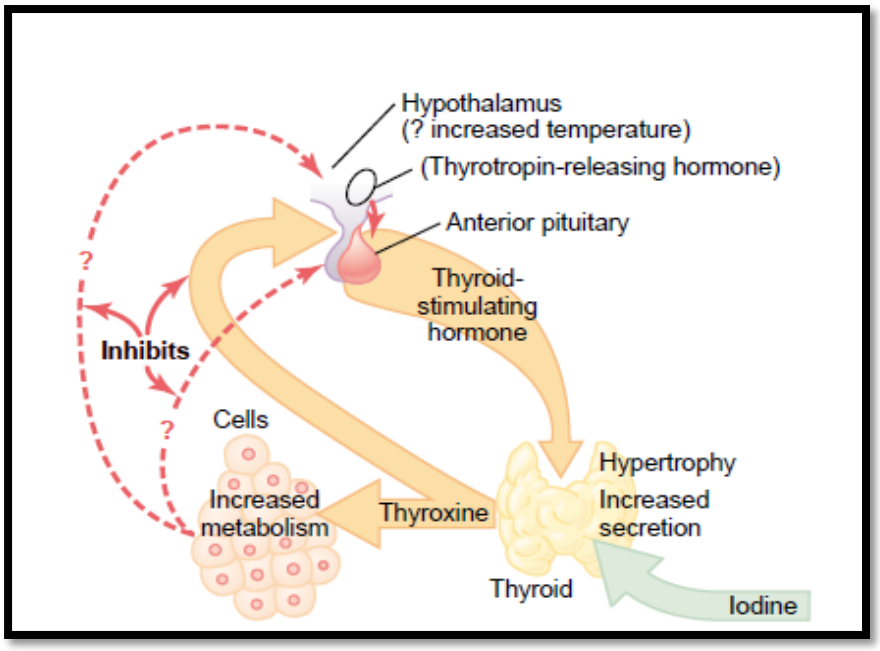


Figure (2-3) : Regulation of Thyroid Secretion (Arthur, 2006).

٢,٥,٤ Thyroid Hormone Action and Metabolism.

The hypothalamic-pituitary-thyroid (HPT) axis controls the amount of Thyroid hormone (TH) in the blood (Mendoza *et al.,* 2017). Thyroid-stimulating hormone (TSH) is released by the anterior pituitary gland in response to hypothalamic thyroid-releasing hormone (TRH), Thyroxine (T4) which has a relatively low affinity to the TH nuclear receptors and triiodothyronine (T3) that most active form of TH are produced in tandem with TSH stimulation in the thyroid (Gereben *et al.,* 2008).

There are several mechanisms that control the movement of TH across cellular membranes, intracellular T4 to T3 conversion or inactivation, interaction of active T3 with nuclear thyroid hormone receptors (TRs), and DNA binding of these TRs , mediate the fine regulation of the biological action of THs, when these pathways are combined, they lead to the transcriptional control of a number of TH target genes (Vissers *et al.,* 2008).

Notably, TH signaling also occurs through non-genomic mechanisms, such as connecting with a membrane integrin v3 and interacting with cellular proteins, nuclear TRs regulate these traditional genomic routes for TH function, the content of TH in target cells is regulated by three iodothyronine deiodinase enzymes (D1, D2, and D3) that are part of the selenocysteine-containing enzyme family and contain a highly conserved active site with the critical residue being selenocysteine amino acid (Arrojo and Bianco, 2011).

Claim that these enzymes cause the removal of one iodine atom from the phenolic ring (activation pathway) or the tyrosyl ring (inactivation pathway) of T4 and T3 in order to metabolize TH (Gereben *et al.,*2008).

To be more precise, D1 and D2 catalyze the conversion of T4 to T3 by discarding an iodine residue from the outer (phenolic) ring of thyroxine, so although D3 is the physiological terminator of TH activity and catalysing the inactivation of T3 by deiodinating the inner (tyrosyl) ring of T4, resulting in

inactive metabolites like T2 or rT3 Dent Deiodinases D1, D2, and D3 are all membrane-integral proteins; however, D1 and D3 are found in the plasma membrane, whereas D2 is present in the endoplasmic reticulum (Gereben *et al.,* 2008).

T3-TRs complexes bind certain TH response elements (TREs) inside chromatin to increase or decrease the expression of target genes, through interactions with TRs, thyroid hormone signaling in target cells is produced (Flamant *et al.,* 2017). The THRA and THRB genes, which respectively encode Thra and Thrb, are the two isoforms of TH receptors (Brent, 2012).

Last but not least, iodothyronines must be transported across the cell membrane for intracellular THs activity to take place, this process cannot be carried out passively but rather call on particular TH transporters, OATP1C1, MCT8, and MCT10 are three transporters that exhibit a high specificit for iodothyronines among those that mediate TH inflow and efflux (Vissers *et al.,* 2008).

Thyroid Hormone has profound effect on many physiologic processes, such as development, growth in children and metabolism (Ribeiro, 2008). Thyroid hormones stimulate diverse metabolic activates in most tissues ,leading to an increase in basal metabolic rate, one consequence of this activity is to increase body heat production, which seems to result, at least in part from increased oxygen consumption and rats of ATP hydrolysis(Choksi *et al.*, 2003).

The specific metabolic effects of thyroid Hormones include:

1) Oxidative Metabolism. 2)lipid Metabolism.

3)Carbohydrates metabolism. 4) Protein metabolism (Stipanuk, 2000).

Thyroid hormones increase the transcription of large numbers of genes the general effect of thyroid hormone is to activate nuclear transcription of large numbers of genes; therefore, in virtually all cells of the body great numbers of protein enzymes, structural proteins, transport proteins, and other substances are synthesized, the net result is generalized increase in functional activity throughout the body , thyroid hormones activate nuclear receptors, the thyroid hormone receptors are either attached to the DNA genetic strands or located in proximity to them shown in (Figure 2-4) (Arthur, 2006) .

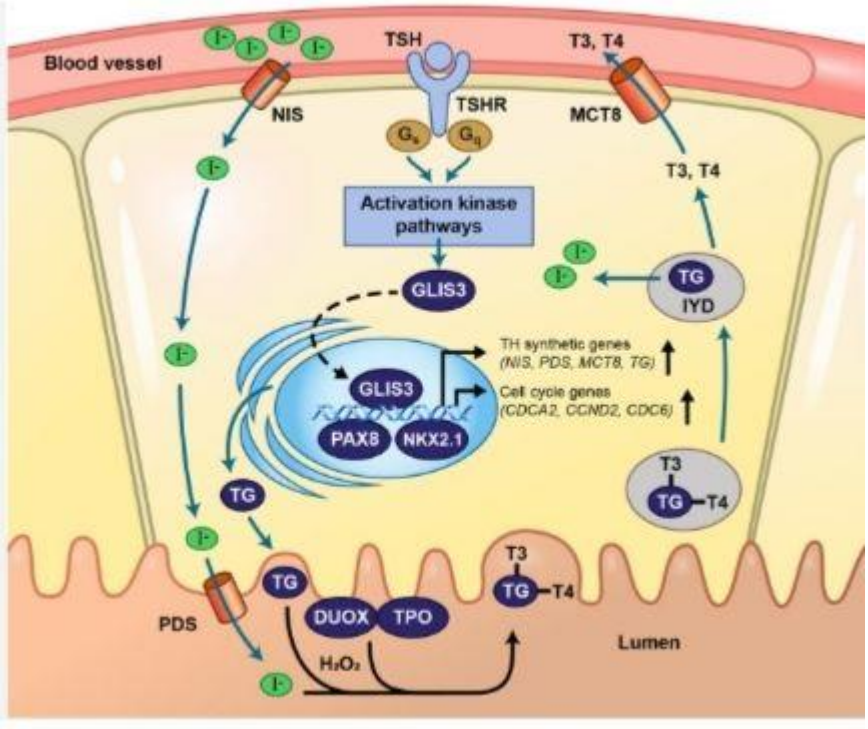


Figure (2-4): Mechanism of Thyroid Hormones Synthesis (Arrojo and Bianco, 2011).

2.5.5 Iodine and Thyroid Gland.

The thyroid gland produces thyroid hormone (TH), which is the primary physiological function of iodine (Zimmermann, 2009 & Sorrenti *et al*,2021) . Sodium/iodide symporter (NIS) actively transports bloodstream iodide through the plasma membrane into the cytoplasm of thyrocytes by exploiting the Na+/K+ -ATPase transporter's concentration gradient as a propulsion source (Wapnir e*t al*.,2003).

Then, a number of transporters, including PENDRIN, ANO1, and CFTR, deliver iodide to the thyroid follicles' lumen (Twyffels *et al.*,2014). Thyroid peroxidase (TPO) utilizes H2O2 generated by DUOX2 to oxidize iodide to iodine radicals and integrates it on particular tyrosine residues inside thyroglobulin (Tg) molecules released by thyrocytes , where it initiates the manufacture of THs ,then TPO binds one monoiodotyrosine (MIT) to one DIT and two residues of diiodotyrosine (DIT) to produce triiodothyronine (T3), and it couples two residues of diiodotyrosine (DIT) to create thyroxine (T4) in the colloid of the follicular lumen, mature Tg that contains THs is kept in reserve , Tg must be reabsorbed from the lumen via micropinocytosis in order for THs to be secreted, and then it must be proteolyzed by lysosomal enzymes in order for THs to be released from the Tg protein (Carvalho and Dupuy,2017).

Iodotyrosine dehalogenase (DEHAL1) is a transmembrane protein that is mostly found in the apical pole of thyrocytes and is involved in the intrathyroidal recycling of iodide, it deiodinates uncoupled MIT or DIT residues (Gnidehou *et al.*, 2004).

Monocarboxylate transporter 8 (MCT8) is primarily responsible for moving THs outside of the basolateral membrane of thyrocytes, where they exit and enter the circulation, the principal regulator of THs production is the pituitary thyroid stimulating hormone (TSH), which, through its receptor (TSHR) located on the basolateral surfaces of thyrocytes, regulates the expression of the thyroid-specific genes involved in TH production (De la Vieja *et al*.,2018). Iodine shortage harms human growth and development in a number of ways (WHO. UNICEF. ICCIDD,2020-2023).

Basil S. Hetzel initially used the term "iodine deficiency diseases" (IDD) some 40 years ago, realizing that the harmful effects of inadequate dietary iodine consumption went far beyond simple goiter (Sorrenti *et al*., 2018).

Important reproductive changes brought on by hypothyroidism as a result of iodine shortage in women include anovulation, decreased fertility, gestational hypertension, stillbirths, congenital abnormalities, and increased perinatal mortality , this might have cultural and social repercussions that compromise the quality of life for parents who must care for a kid who has major health issues (Ferri *et al*.,2003; Koyyada and Orsu, 2020) .

From a physiological perspective, it is possible to interpret infertility in iodine-deficient hypothyroid women as a defense mechanism set up by the body to prevent risks associated with pregnancies carried out under iodine deficiency conditions (Mills *et al*.,2018).

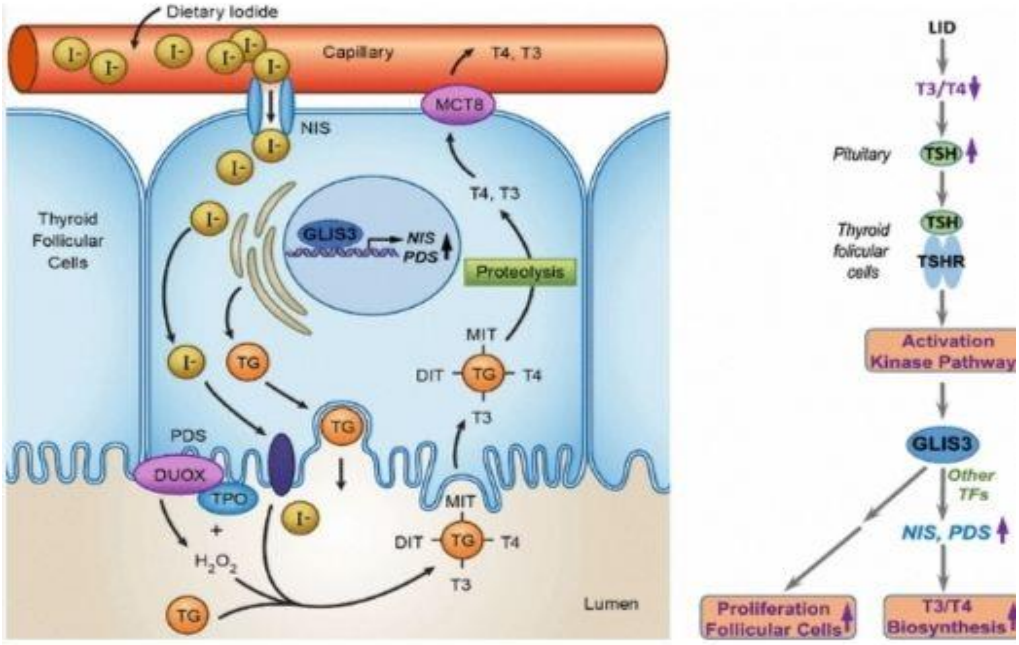


Figure (2-5): Mechanism of Role Iodine in Thyroid Hormones Synthesis (Rebecca *et al.*, 2011).

2.5.6 Effect the High Intake Iodine on Thyroid.

The production of the thyroid hormones T4 and T3 is iodine's primary role in metabolism, the deiodinating enzymes deiodinase 1, 2, and 3 (D1, D2 and D3), which are found in the liver, kidney, and numerous peripheral organs where they help to maintain physiologically active T3 concentrations, are essential for the biological activity of thyroid hormones (Peeters *et al.*, 2017).

Iodine must be consumed since it cannot be produced by the body and must be obtained from food or environmental sources. Iodine is absorbed by the stomach and is then transported by the blood to the thyroid and several extrathyroidal organs (Smyth *et al*.,2014).

A kind of hyperthyroidism known as Jod Basedow may develop in people with autonomously functioning nodules, possibly as a result of a past iodine shortage situation, treatment with the cardiac antiarrhythmic medication amiodarone (contains 75 mg iodine per pill) can cause amiodarone-induced thyrotoxicosis (AIT 1) or AIT 2, which is characterized by destructive thyroiditis (Bartalena *et al.*,2018).

Other sources of excessive iodine consumption include food colorings like erythrosine, nutritional supplements like kelp pills, and occasionally cough mixtures, though many of these are now iodine-free (Farebrother *et al.*,2019).

With dietary iodine restriction frequently encouraged, high iodine levels can reduce the efficacy of RAI treatment for thyroid cancer or hyperthyroidism ,not all excessive iodine consumption has negative effects, KI or KIO3 tablets with a high iodine concentration (about 100 mg) are frequently given as a protective blocking

agent to people at danger of swallowing RAI resulting from a nuclear disaster, one might infer that the effects of Radioactive Iodine (RAI) uptake and future thyroid cancers following the Fukushima disaster in 2011 would have been substantially worse in a population other than Japanese people who consume seaweed and have access to a lot of iodine (Braverman *et al*.,2014).

Variations in dietary iodine consumption can encourage thyroid cells proliferation in thyroid cancer both low and high dietary iodine intake can lead to an increase in blood TSH (Zimmermann *et al*., 2015).

locations with both low and high dietary iodine consumption have been linked to higher risks of thyroid cancer, study relating changes in differentiated thyroid cancer (DTC) rates to iodine consumption are complicated by a number of variables, including ethnicity, goitrogen and selenium intake or carcinogen intake, japanese postpartum women who regularly eat seaweed dishes have been shown to have an elevated risk of papillary thyroid cancer compared to those who only eat it twice a week or less (Michikawa *et al*.,2012).

Long-standing research links iodine shortage to the etiology of thyroid malignancies, specifically follicular thyroid carcinoma and maybe the anaplastic variety , whatever the cause of the reduced or increased iodine intake, any reduction in the production or release of thyroid hormones would certainly result in a rise in TSH and a stimulant for unfavorable thyroid cells development (Zimmermann *et al*.,2015).

2.5.7 The Transcription Factors of Thyroid Gland.

The thyroid gland functional unit, the follicle that results from differentiated thyroid epithelial cells forming follicular structures around a lumen, is known as the thyroid gland , more recent research has shown that the thyroid's functional unit is made up of more complicated components than previously believed, in this regard, the idea of an angiofollicular unit—which is created during embryonic development and also comprises endothelial cells of the blood arteries that surround the thyroid follicles—has been put up (Colin *et al*., 2004).

These four genes—Nkx2-1 (previously known as thyroid transcription factor 1, TTF1 or T/ebp), Foxe1 (formerly known as thyroid transcription factor 2, TTF2), Pax8 and Hhex (hematopoietically expressed homeobox protein)—are expressed by differentiated follicular cells show as Figure (2-6) (Nilsson and Fagman , 2017).

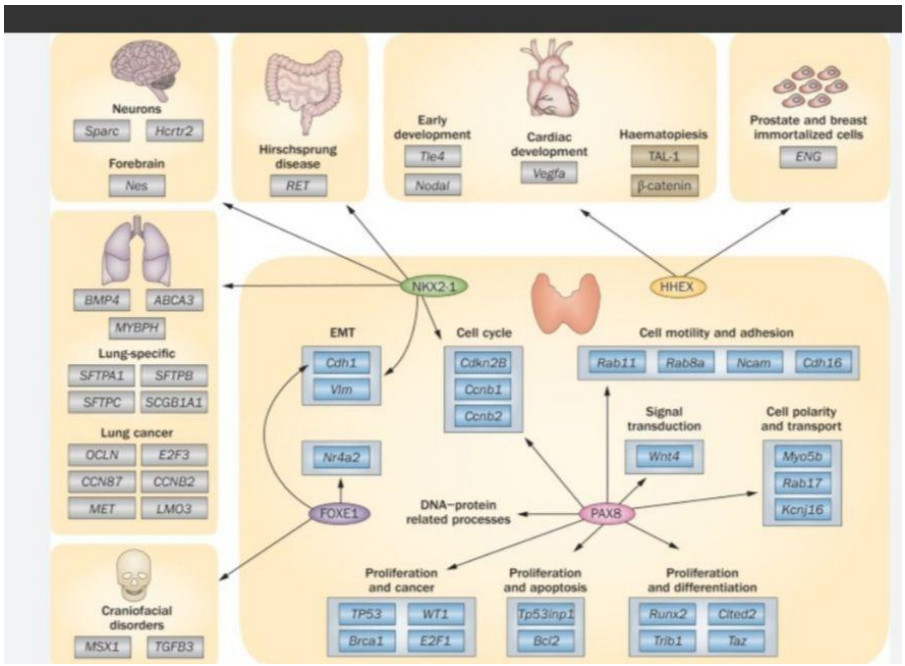


Figure (2-6): Transcription Factors of Thyroid Gland (Nilsson Fagman , 2017).

2.5.7.1 Homeo Box Protein (Nkx 2-1):

Thyroid transcription factor-1 (TTF-1), is a 38KD home domain referred to as thyroid-specific enhancer binding protein (NKx2.1), is tissue-specific transcription factor with a protein folding structure that is primarily expressed in differentiated cells derived from the foregut endoderm and neuroectoderm, including thyroid follicular cells and type II alveolar epithelial cells, TTF-1 controls the expression of important functional genes in the thyroid show as Figure (2-7) (Phelps *et al*.,2018 ; Oktay *et al*.,2020).

NKx2-1/TTF-1 expression in thyroid and lung during human development including differentiation and morphogenesisof both thyroid and lung , the mechanisms that control Thyroid-stimulating hormone (TSH), nuclear factor (NFI), and the binding site of TTF-1 themselves all control how much of TTF-1 is expressed (Nilsson and Fagman , 2017) .

Most mutations in the TTF-1 gene, including insertion mutations, heterozygous mutations, and deletion mutations, affect the homologous domain of the gene, thyroid cancer develops because the mutant TTF-1 is unable to attach to the target DNA that losing its ability to regulate thyroid cell differentiation and maturation and potentially inducing malignant transformation, among them families with papillary thyroid cancer and multinodular goiter have been shown to have missense mutations in the TTF-1 gene that cause an alanine to valine substitution at codon 339 (Ngan *et al.*,2009).

Follicular thyroglobulin (TG) can lower the amount of NFI mRNA and protein, particularly the amount of NFI-A protein, as well as lower the binding between TTF-1 and NFI, which also controls how much of TTF-1 is expressed, when TSH binds to the thyroid-stimulating hormone receptor on a cell's surface, it connects with the G protein's alpha subunit and increases cAMP, which in turn stimulates PKA and raises the production of TTF-1(Colin *et al*., 2004).

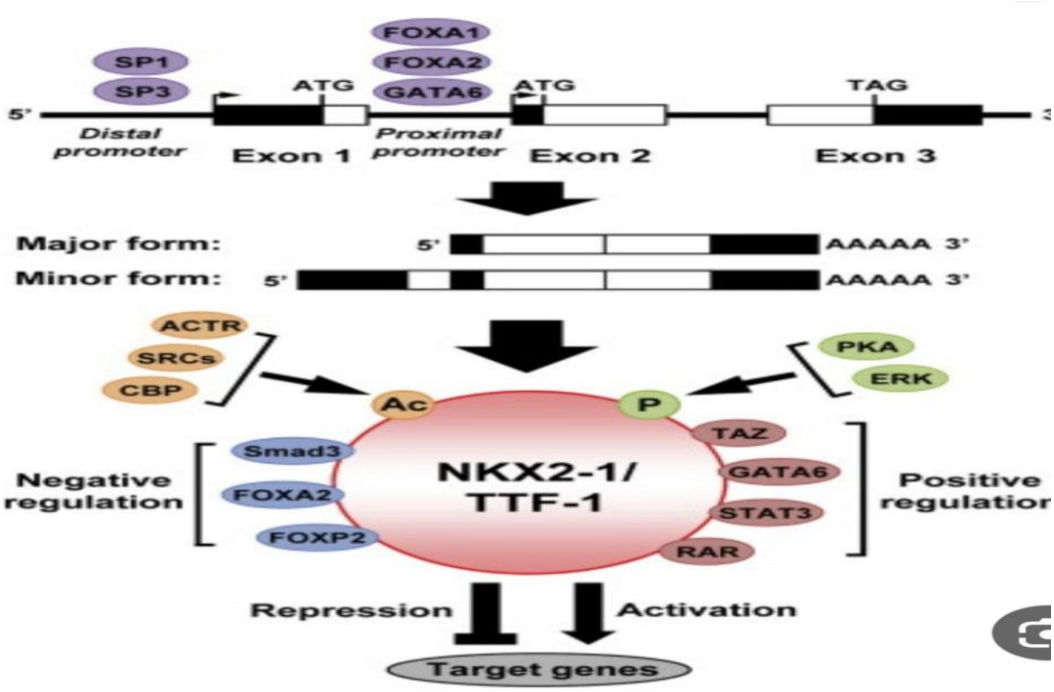


Figure (2-7): Function of Homeo Box Protein (Nkx 2-1) (Oktay *et al*.,2020).

2.5.7.2 Forkhead Box Protein E1(FOXE-1) :

On chromosome 9q22, the human FOXE1 gene, formerly known as thyroid transcription factor-2, codes for a DNA-binding protein that belongs to the family of evolutionarily conserved transcriptional regulators known as the forkhead/ winged-helix family (Cuesta *et al.*, 2007).

This transcription factor possesses a polymorphic polyalanine (poly-A) tract immediately distal to its DNA-binding domain (rs71369530), which fluctuates between 11 and 22 alanine residues, despite FOXE114Ala and FOXE116Ala accounting for more than 98 percent of known alleles (Kallel *et al*., 2010).

A crucial component of thyroid organogenesis, the transcription factor FOXE1 is unique to the thyroid and is required for thyrocyte precursor migration throughout the early stages of thyroid development , in addition to being essential for the thyroid gland correct development, FOXE1 coordinately maintains the thyroid gland differentiated status together with PAX8 and NKX2-1 (Fernandez *et al*., 2015).

In the differentiated thyroid, the transcriptional activator Foxe1 modulates the ability of cells to react to extrinsic stimuli including thyroid stimulating hormone, insulin-like growth factor-1, and transforming growth factor- (Lopez-Marquez *et al*., 2019). In a rat thyroid follicular cell line that also included Cdh1 and Nr4a2, our team recently identified two thyroid-specific genes (Duox2 and Slc5a5) as new Foxe1 targets show as Figure (2-8) (Fernandez *et al.,* 2013).

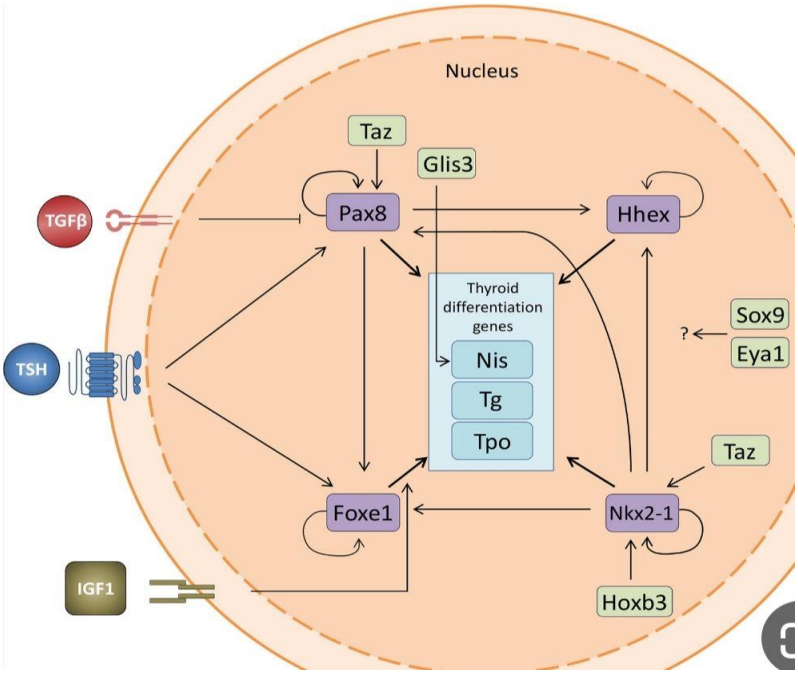


Figure (2-8): Function of Forkhead Box Protein E1(FOXE-1) (Fernandez *et al.*, 2013).

2.5.7.3 Paired Box Gene 8 (PAX-8):

The PAX 8 genes, which belong to the paired-box family, code for transcription factors with DNA-binding paired-box domains , based on the existence of an octapeptide region, paired-type homeodomain, or both, they have been further subclassified into four categories (I-IV) (Blake and Ziman, 2004).

These transcription factors avoid functional defects in some cells after birth and are essential for organogenesis throughout embryonic development , the PAX8 gene was primarily discovered to have a significant role in the development of the thyroid and kidneys (Laury *et al.*, 2011).

In a few research, the peroxisome proliferator-activated fusing (PPFP) influence on PAX8 function has been evaluated the genes for thyroid-stimulating hormone receptor (TSHR), thyroid peroxidase (TPO), and thyroglobulin (Tg) promotors are all regulated by PAX8, the interaction between PPFP and PAX8 is complicated (Placzkowski *et al.,* 2008) . PPFP upregulates the expression of NIS and TPO while downregulating the expression of TSHRs and Tg show as Figure (2-9) (Tan *et al.*, 2021).

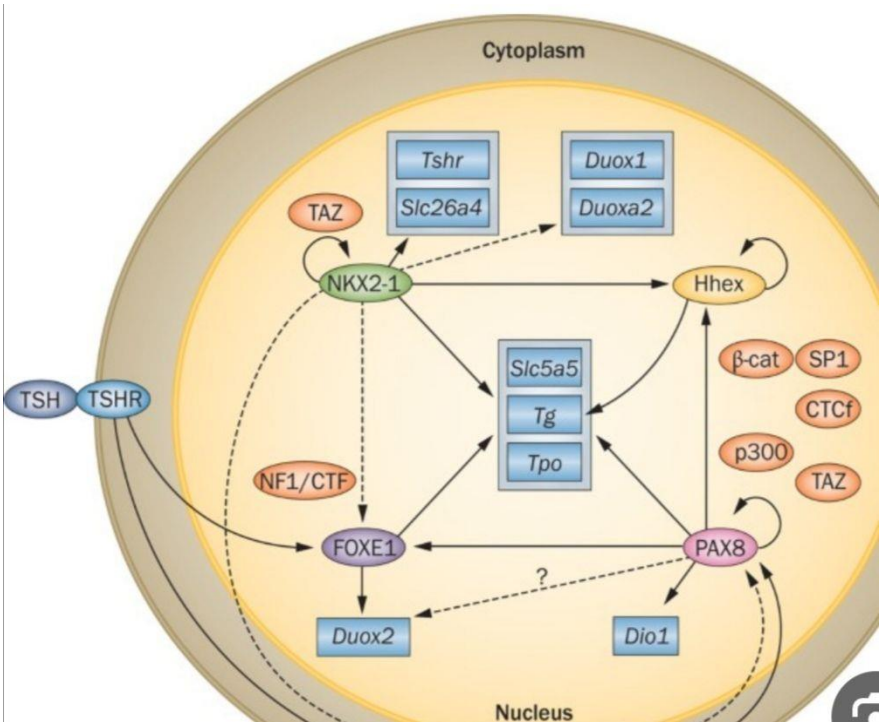


Figure (2-9): Function of Paired Box Gene 8 (PAX-8) (Tan *et al.*, 2021).

2.6 The Brain Structure and Histology.

The brain is a nervous system organ that controls senses, movement, emotions, language, reasoning, and memory in addition to task-evoked reactions, one of the following brain organs is the cerebrum: it divides into the left and right hemispheres of the brain (Lenroot and Giedd,2006) .

The hemispheres of the brain have wrinkled and folded surfaces, the ridges that may be seen in between the convolutions are called gyri, the valleys between the gyri are referred to as sulci (plural of sulcus) fissures are the name given to deep sulci the grey tissue that lines the exterior of both cerebral hemispheres is known as the cerebral cortex (Maldonado *et al*.,2022).

The cerebellum is made up of the cerebellar cortex and deep nuclei the cerebellar cortex is made up of three layers: (granular, Purkinje, and molecular) these structures are known as the cerebellar peduncles, which join the brainstem to the cerebellum, controlling balance, posture, and motor coordination is the cerebellum's primary function (Budday *et al.*, 2015).

The brainstem It contains the midbrain, pons, and medulla are all found in the brainstem, it's situated between the spinal cord and the brain's base (Maldonado *et al*.,2022).

2.6.1 Basics of Early Brain Development.

During the fast expansion of early brain development, the essential building blocks of the brain's structure are developed (Knickmeyer *et al.*,2008) . From gestation to old age, there are three phases in the development of the brain, cortical thickness (CT) and surface area (SA) expansion are caused by cell migration and proliferation throughout the first half of pregnancy , halfway during gestation, the second stage starts, and it lasts for up to two years (Budday *et al.,* 2015) .

New neural connections predominate, leading to tangential growth of the outer cortex and cortical tension that causes gyrification , myelination reaches its apex, causing more white matter to grow, the remaining portion of a person's lifespan is dedicated to the last phase of brain development, which mostly entails synaptic pruning and even some synaptogenesis due to the cortex's ongoing plasticity, gyrification can take place to compensate for the cortical stress brought on by the sparse neural connections formed at this time, the variety of neuronal progenitor cell types play a major role in regulating and supporting normal brain development (Lenroot and Giedd,2006) .

Cell proliferation results in fast CT and SA growth during the first two years of life, with CT typically reaching 97% of adult values and SA reaching 69%, radial glial cell (RGC) division in the ventricular zone results in progenitor cell generation that is exponential, which raises CT and SA 2 levels, A single intermediate progenitor cell (IPC) and an RGC that stays in the ventricular zone are produced when RGCs divide asymmetrically (Budday *et al.*,2015) .

IPCs fill the cortical layers radially whereas RGCs migrate tangentially to enlarge the cortex, According to the theory of the radial units, the expansion of SA is mostly caused by the number of radial units, whereas thickness is caused by the neuronal output from the radial units (Pontious *et al.*,2008) .

Increases in synapses, dendrites, long-distance axons, and myelination are the causes of cortical structural expansion (Gao W *et al*., 2017) . For the integration and effective execution of certain brain processes, the fundamental organization of the neural circuitry must be properly established (Lehtinen and Walsh, 2011).

However for those who are at risk and those who have schizophrenia, changes in cognition, gray matter, white matter, CT, and cerebrospinal fluid (CSF) are distinguishing structural features , we can discover cognitive delays and changes in brain structure during fast brain growth by using MRI data and cognitive evaluations to determine a potential time of intervention as Figure (2-10) (Gao X. *et al*., 2017).

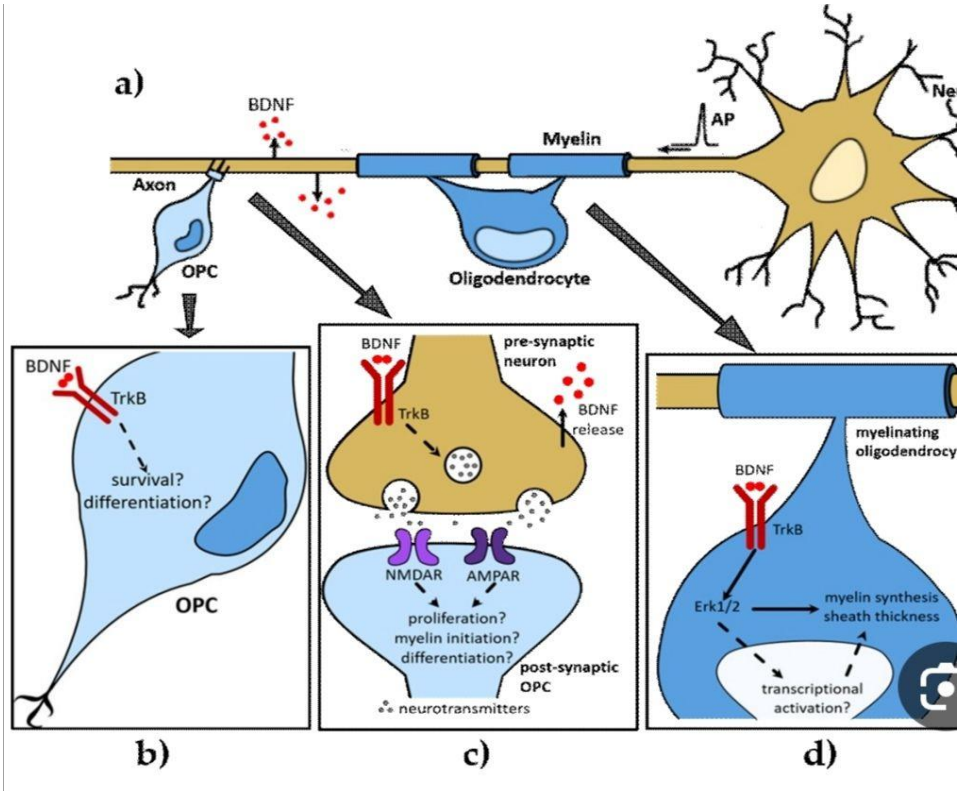


Figure (2-10): Neurotransmitter (Gao X. *et al*., 2017).

2.6.2 The Brain Neurotransmitter Factors.

2.6.2.1 Acetylcholine(Ach):

Acetylcholine(Ach) a neurochemical, is used by the brain and other organ systems of the body to carry out a variety of functions, it is a neurotransmitter, more precisely, a chemical messenger that permits communication between neurons and other specialized cells like myocytes and cells in glandular tissues , it is generated by neurons as Figure (2-11) (Waxenbaum *et al*.,2021).

ACh is a neurotransmitter that is present throughout the body, although it is most often found at the neuromuscular junction, at the neuromuscular junction, motor neurons in the ventral spinal cord link to muscles throughout the body to activate them , ACh performs a variety of functions in the autonomic nervous system, including acting as a neurotransmitter between preganglionic and postganglionic neurons (Salazar *et al*.,2019).

ACh serves as a neurotransmitter in the autonomic nervous system as well, functioning as the ultimate release product from parasympathetic postganglionic neurons as well as the neurotransmitter between preganglionic and postganglionic neurons (Waxenbaum *et al*.,2021).

ACh production is increased by the hormone thyrotrophic-releasing hormone (TRH) (Annerbo and Lokk, 2013) . According to one study, the amount of acetylcholine in the hippocampi of people with hypothyroidism had dramatically reduced and that T4 treatment brought ACh levels back to normal (Wang *et al*.,2014) .

Hyperthyroidism can be brought on by a lack of iodine can adversely impact the brain's capacity to produce Ach influencing mood as well as cognition, memory, learning, and recall the neurotransmitters in the brain are adversely impacted by inadequate iodine intake that can lead to neurodegenerative disorders like Alzheimer's and Parkinson's as well as depression, mental fog, anxiety, learning, and memory issues (Zimmermann, and Boelaert, 2015 ; Salazar *et al*.,2019).

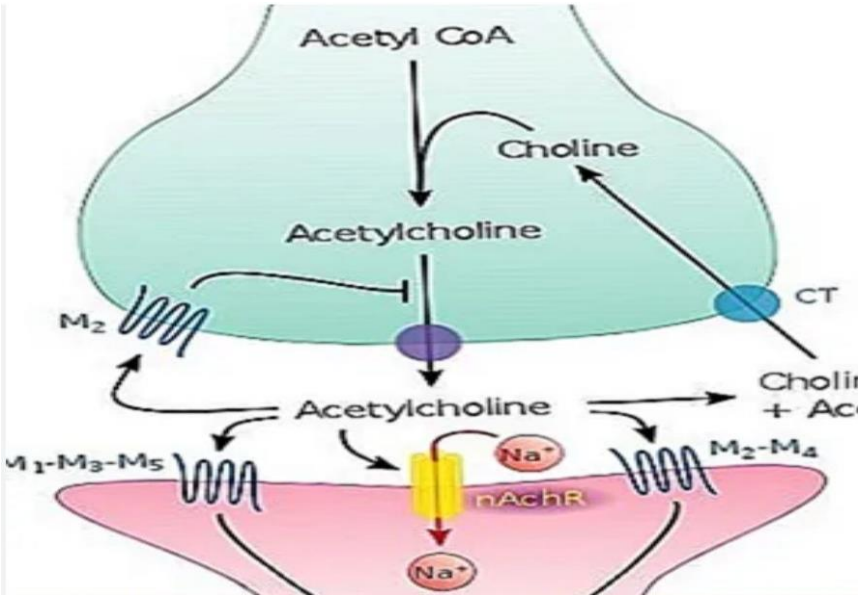


Figure (2-11): Function of Acetylcholine(Ach) (Waxenbaum *et al*.,2021).

2.6.2.2 Brain-Derived Neurotrophic Factor (BDNF) :

The well-known neuronal growth and differentiation regulator brain-derived neurotrophic factor (BDNF) also controls neurotransmission in CNS synapses (Castrén and Antila ,2017) . The three compartments of neuromuscular junctions are axon terminals, perisynaptic Schwann cells, and muscle fibers (NMJs) that have been proven to contain BDNF and its receptors (Wang *et al.*, 2022) .

Additionally, because it may be released from muscles and function as a retrograde messenger on nerve terminals, BDNF is currently regarded as a myokine (Gaydukov *et al.*, 2019).

Recently, it became clear that BDNF's many synaptic actions cannot be understood well without considering the potential simultaneous action of its maturation by-products (Bogacheva *et al.*,2022) .

It is unknown if autoimmune thyroiditis (AIT), which can be brought on by too much iodine, has any effects on neurodevelopmental processes in children that due to the knowledge that brain-derived neurotrophic factor (BDNF) plays a significant role in neurodevelopment, we investigated the impact of experimental autoimmune thyroiditis (EAT) rats with varying iodine consumption on offspring brain development as finger (2-12) (Jin *et al.*,2022) .

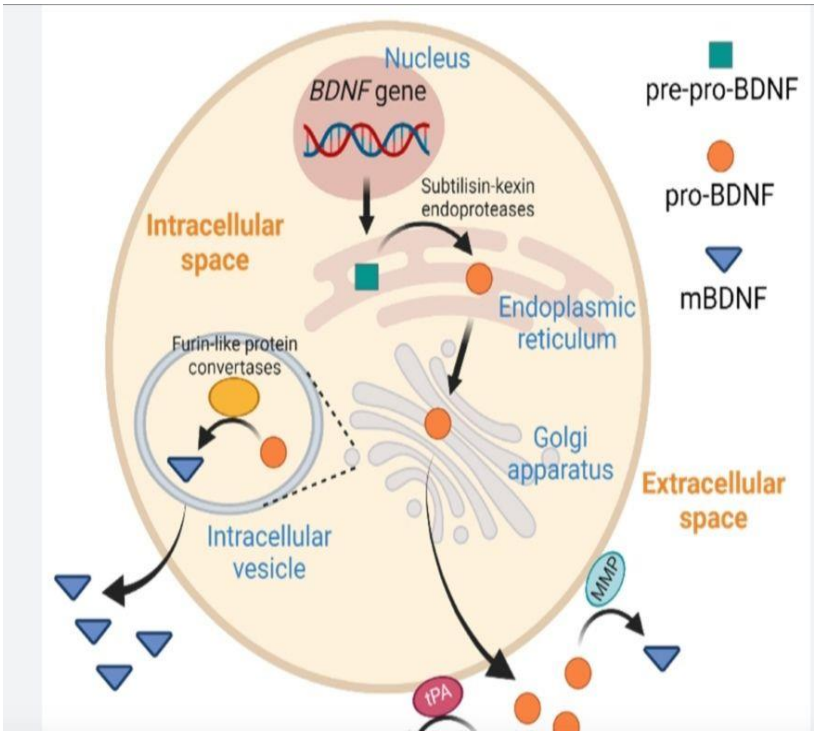


Figure (2-12): Function of Brain-Derived Neurotrophic Factor (BDNF) (Jin *et al.*,2022).

2.8 Effect Iodine of Body Weight.

Iodine is a crucial component of the thyroid gland hormones and is necessary for normal physical growth during pregnancy and infancy, the ability to synthesize thyroid hormones is compromised when dietary iodine needs are not satisfied, growth and development may be hampered by the hypothyroidism that results from iodine deficit throughout pregnancy, childhood, and early childhood (Andersen and Laurberg, 2016).

Iodine deficiency decreases the action of thyroid hormones, which in turn decreases the effects of growth hormone (Shahid *et al*.,2022) .Either at its receptor or through effects on pituitary production, as well as circulating levels of insulin-like growth factor (IGF-1) and its binding proteins (Pesce and Kopp, 2014).

Iodine deficiency disorders (IDD) are a spectrum of developmental and functional problems that can be brought on by iodine shortage and represent a global health threat to individuals and societies , IDD prevention programmes have been introduced in many parts of the world (Völzke *et al*.,2018).

In 98 premenopausal obese women (BMI > 30 kg/m2) and 31 age-matched non-obese women (BMI 25 kg/m2) from a mild to moderately iodine deficient area, it was shown that obese women had higher goiter prevalence (24.5 percent vs 12.9 percent) and increased thyroid volume (15.2 5.3 mL vs 12.2 3.3 mL), in addition, a > 10% weight loss after 6 months of obesity medication resulted in a considerable decrease in thyroid volume, which was mirrored by a similar significant decline in TSH levels (Sari *et al*., 2003).

Traditionally, hypothyroidism has been related to obesity whereas hyperthyroidism has been connected to underweight people. but just a small number of research have examined these connections (Fouad Z. Hamza and Muna H. AL-Saeed , 2021).

2.9 Effect Iodine of Reproduction.

Although there is considerable curiosity in how thyroid hormones (THs) affect female reproductive, the mechanism is yet unknown, the impact of TH dysregulation on rat reproductive hormones was examined by (Liu *et al.,* 2018) . Rats were given L-thyroxine and propylthiouracil (PTU) to produce hypo- and hyperthyroidism decreases the frequency and amplitude of spontaneous rhythmic contractions in the uterus and puberty was markedly delayed of virgin rats and lead to reduced endometrial and myometrial volumes and uterine weight that raise levels of abortions or less chance of pregnancy(Parija *et al*., 2001).

The role of Iodine and that effect on levels of thyroid hormones(TH) that mean maternal hyperthyroidism and TH dysregulation affects the profiles of reproductive hormones especially estrous cycle also estrogen and progesterone stimulated liver for product thyroglobulin binding proteins, and reduces capacity to ovulate and form corpora lute by reducing the population of resting oocytes, the number of activated follicles, and the primordial follicle pool, also THs effect on human chorionic gonadotropin hormone (HCG) , it stands to reason that maternal hyperthyroidism would be linked to lower fertility rates (Parija, *et al.*, 2006; Bagheripuor *et al.,* 2018).

3.1.Design of study

3.1.1 Experimental Design

Experiment - control study was conducted between April 2021- January 2022 and it was carried out at the Animal House of the Biology department / College of Science at the University of Babylon. The study included (95) laboratory rats with weights ranging 250-300 gm and 8 weeks of life were used in the experiment ,with environmental conditions that include moderate temperature included (20 males, 75females), 15 females as a control group, and 60 females dosed with iodine , and 15 males it were used for intercourse only without being dosed .

The animals were treated with the approval of the ethics committee at the department, where they were kept in meshed plastic cages(in 45cm×27cm×16cm polypropylene and covered with metal mesh, and the floor covered in sawdust) containing sawdust to give pellets and water with cleaning the animals by changing sawdust once a week and sterilizing the cages under temperature (25 °c) throughout the experiment. The animals were left to adapt for 15 day before starting the experiment with treated females by different iodine doses for 60 day after that male added for 15 day and checking the female every day for pregnancy by mucus ring with record food intake **,** mothers weight and mortality rate of mothers**.**

This study was included 5 groups as following:

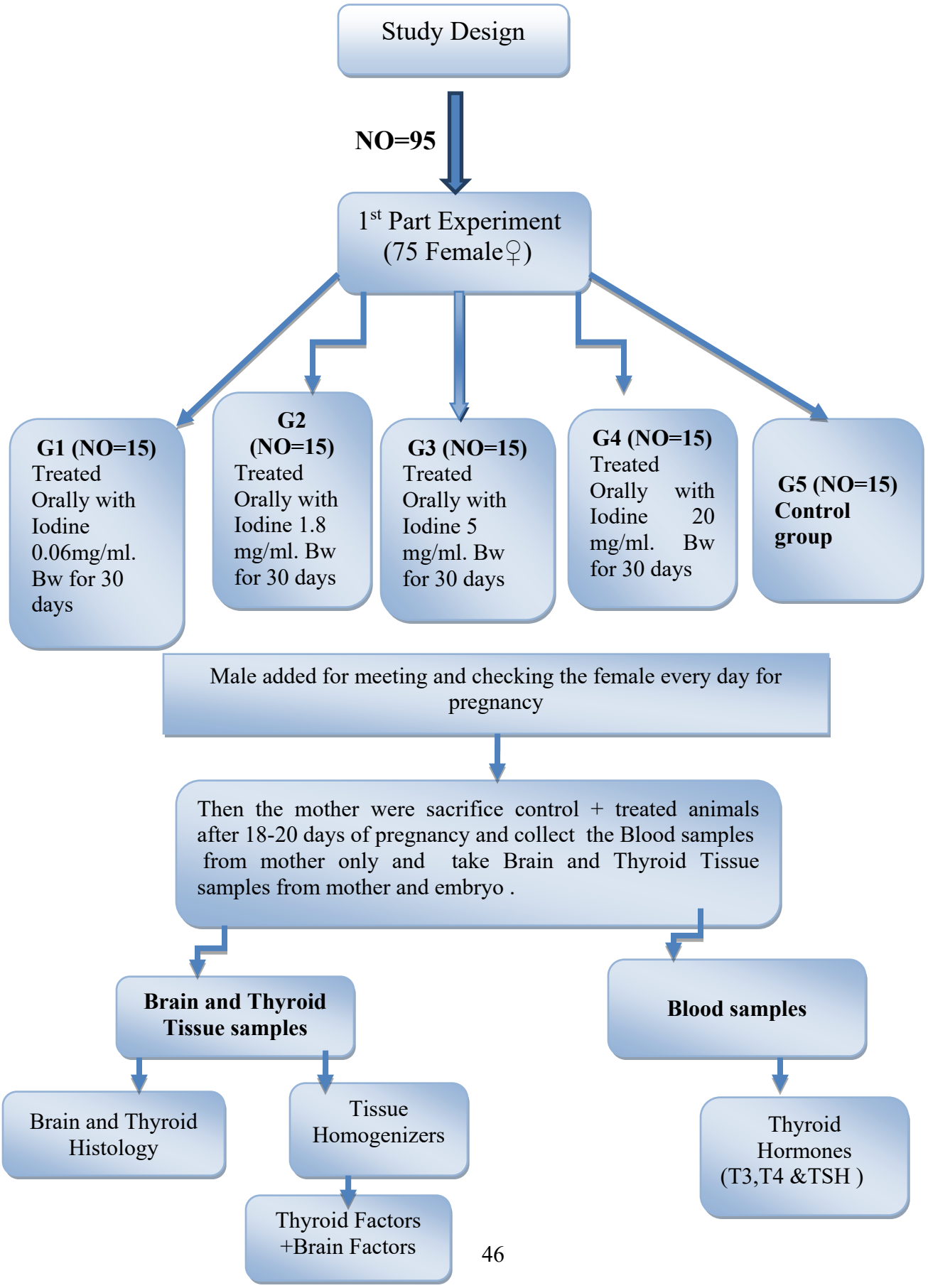
Group (1): Involved 15 adult female rats treated with dose of iodine (0.06 mg /ml)

Group (2): Involved 15 adult female rats treated with dose of iodine (1.8 mg/ ml)

Group (3): Involved 15 adult female rats treated with dose of iodine(5mg /ml)

Group (4): Involved 15 adult female rats treated with dose of iodine(20mg /ml)

Group (5): Involved 15 adult female rats, animals in this group administered distilled water and served as control.


Study Design
NO=95
1st Part Experiment (75 Female♀)
G1 (NO=15) Treated Orally with Iodine 0.06mg/ml. Bw for 30 days
G2 (NO=15) Treated Orally with Iodine 1.8 mg/ml. Bw for 30 days
G3 (NO=15) Treated Orally with Iodine 5 mg/ml. Bw for 30 days
G4 (NO=15) Treated Orally with Iodine 20 mg/ml. Bw for 30 days
G5 (NO=15) Control group
Male added for meeting and checking the female every day for pregnancy
Then the mother were sacrifice control + treated animals after 18-20 days of pregnancy and collect the Blood samples from mother only and take Brain and Thyroid Tissue samples from mother and embryo .
Brain and Thyroid Tissue samples
Blood samples
Brain and Thyroid Histology
Tissue Homogenizers
Thyroid Hormones (T3,T4 &TSH)
Thyroid Factors +Brain Factors

3.1.2 Preparation iodine

Iodine powder by weighted of an electronic balance, the doses were dissolved in distal water every day over the experiment period . The solution was administered orally for females by gavage in a dose after conducting an experimental dose to measure the safety of the dose on one animal for 24 to 48 hr before use.

3.1.3 Blood Samples

All animal groups were sacrifice after 18-20 days of pregnancy. Gage (A G) 23 needle was used to take three milliliters of blood from the heart from mother only , and the remaining blood was allowed to clot at room temperature in a gel test tube. After 15 minutes of centrifugation at 2500 Rpm , the serum was aspirated, divided into aliquots, and kept at -20C until hormone levels(T3,T4 and TSH) could be determined .

3.1.4 Preparation Tissues Samples

3.1.4.1 Tissues Homogenization

The actual technique of grinding is rather straightforward and include introducing an extraction buffer and the tissue to the homogenizer tube, then carefully pressing the pestle on to the sample with a twisting motion. All groups of animals were euthanized after 18-20 day after the pregnant take Brain and Thyroid Tissue samples from mother and embryo .To spin the sample and expose all surfaces to grinding, the piston is lifted and lowered while being turned. This is done once more until Brain and Thyroid factors levels(NKX2-1, FOXE1 and PAX-8) could be determined .

.31.4.2 Histological

The thyroid gland and the brain for mother and embryo were weighed with electronic balance and taken in a container containing formalin37% for histological study.

3.2.Materials

3.2.1 Solutions and Reagents: All the solutions and reagents used are summarized in table (3-1).

Table (3-1): Solutions and reagents and their suppliers and sources.

No.	Solutions and Reagents	Sources &suppliers
1	Acetylcholine (ACH) ELISA Kit	Diagnostic System Laboratories (DSL)/ USA
2	Brain drived neurotrophic facor (BDNF) ELISA Kit	DRG International, Inc./ Germany
3	Thyrotropin (TSH) ELSA Kit	Monobind Inc. USA
4	Total Thyroxin (tT4) ELSA kit	Monobind Inc. USA
5	Total Triiodothyronine (tT3) ELISA Kit	Monobind Inc. USA
6	Forkhead box protein E1 (FOXE1) Kit	Cusabio USA
7	Homeo box protein Nkx02 (NKX2-1) Kit	Cusabio USA
8	Paired box protein Pax-8 (PAX8) Kit	Cusabio USA
9	Eosin	Sigma Chemical Co. (USA)
10	Hematoxylin	Sigma Chemical Co. (USA)
11	Ethyl-alcoho	Milpharm/London/UK
12	Formalin 10-40%	TEDIA Company INC, USA
13	Paraffin wax	Sungia Petani industrial, Malaysia

3.2.2 Apparatuses and Instruments: Equipment and instruments used in this study are summarized in table (3-2).

Table (3-2): Apparatuses used in the experiment and their suppliers.

No.	Apparatuses	Supplier
1	Elisa	Biokit ELx800/USA
2	Centrifuge-CL008	Cypress Diagnostics/Langdorp-Belgium.
3	Cool Centrifuge	Cecil Instruments/Cambridge- England
4	Refrigerator with freezer-20C	LG/Korea
5	Electrical sensitive Balance	CAMRY/China
6	ThermalConsole(TEC2900)	Histo-line(Italia)
7	Tissue processor(ATP1000)	Histo-line(Italia)
8	Rotary Microtome(NRS3500)	Histo-line(Italia)
9	Embedding center(TEC2900)	Histo-line(Italia)
10	Microscope with computer camera	Measuring 3m/10ft/China
11	Micropipettesand Tips (small, large)	Gilson/ France
12	plane tube	AFCO-DISPO/ Jordan
13	Gel tube	AFCO-DISPO/ Jordan
14	Disposable gloves.	TG Medical /Malaysia.
15	Disposable syringes 5 cc	Medeco® Inject /Abu Dhabi Medical Devices Co.L.L.C.
16	Cotton	NFLB/CHINA
17	Plastic Gage	Local market/Iraq
18	Universal container	Jordan
19	Dissection set	China
20	glass slides	China
21	cover slips	China
22	Insulin syringe	Q-ject China
23	Gavage	German
24	Deep freezer	China

25	plastic containers	China
26	Eppendorf tube	China
27	Water bath	China
28	Oven	China
29	Incubator	China
30	Glass Homogenizers	UK

3.3 Methods

3.3.1 Measurements Of Hormones By ELISA Assay(Enzyme-linked immunosorbent assay)

By identifying the presence of antibodies or antigens in the blood or other bodily fluids, the enzyme-linked immune sorbent assay (ELISA) is a biochemical technique used in the laboratory to assist in the diagnosis of numerous medical disorders.This test is used to determine the concentration of a certain protein or compound, such as infectious pathogens, allergies, hormones, or medications, in blood or urine. This test depends on the interaction of immune system building blocks known as antigens and antibodies. Proteins called antibodies are made by the body to recognize and destroy any potentially harmful foreign invaders, such as bacteria and viruses. Antigens are the chemicals that cause antibodies to be generated because they stimulate an immune response.

3.3.1.1 Determination of Total Triiodothyronine

3.3.1.1.1 Principle

Total triiodothyronine radioimmunoassay (TT3) is a comparison assay. In tubes coated with antibodies, samples and calibrators were treated with 125I_ tagged T3 as a tracer. After incubation, the liquid inside the tubes was aspirated, and a gamma counter was used to measure the bound radioactivity. Unknown values were extrapolated from a standard curve after its construction (lindstedt ,1984; Wolfish, 1981; Wenzel, 1981). The typical T3 range is 0.51 to 1.85 ng/ml.

3.3.1.1.2. Reagents

1. Two hundred and fifty coated tubes of the anti-T3 monoclonal antibody (ready to use).

2. One 22ml vial of 125I_ Labeled T3 Tracer (ready to use). At the time of manufacturing, the vial includes 165 KBq of 125I-labeled T3 in a buffer containing protein, sodium azide (less than 0.1%), and a dye.

3. Calibrators: six 0.5 ml ready-to-use vials containing 0 to 12 nmol/L of T3 in human serum with sodium azide (0.1%) were used as calibrators.

4. Control serum: two lyophilized vials of T3 in human serum served as the control.

3.3.1.1.3 Procedure

Tubes	Total	Calibrator	Control	Sample
Calibrator		25 µl		
Contro			25 µl 25 µl	
Tracer	200 µl	200 µl	200 µl	200 µl
Incubated 1 hour at (18 – 25 ˚C) with shaking (>280 rpm).				
The fluid was decanted and blotted on filter paper.				
Radioactivity was Counted by gamma counter.				
The results were calculated by calibration curve.				

3.3.1.2 Determination of Total Thyroxin

3.3.1.2.1. Principle

Total Thyroxin (TT4) radioimmunoassay is a competitive assay. In tubes covered with antibodies, samples and calibrators were treated with 125I-labeled T4 as a tracer. After incubation, the liquid inside the tubes was aspirated, and a gamma counter was used to measure the bound radioactivity.

Unknown values were extracted from a standard curve by interpolation (Lindsted, 1984; Walfish, 1981; Wenzel, 1981).T4 levels should range from 4.4 to 11.8 mg/dl.

3.3.1.2.2. Reagents

1- Tubes coated with an anti-T4 monoclonal antibody: 2 x 50 tubes (ready to use).

One 55ml vial of 2-125 I Labeled T4 tracer (ready to use). At the time of production, the vial contains 110 KBq of 125I-labeled T4 in a protein- and sodium azide-containing buffer (0.1%).

3-Calibrators: Each of the six 0.5 ml vials (ready to use) included 0 to 400 nmol/L of T4 in human serum along with 0.1 percent sodium azide.

4- Control serum: two lyophilized vials containing lyophilized T4 in human serum served as the control.

3.3.1.2.3. Procedure

Tubes	Total	Calibrator	Control	Sample
Calibrator		20 µl		
Contro			20µl	
Sample			20 µl	
Tracer	500 µl	500 µl	500 µl	500 µl
Incubated 1 hour at (18 – 25 ˚C) with shaking (>280 rpm).				

The fluid was decanted and blotted on filter paper.
Radioactivity was Counted by gamma counter.
The results were calculated by calibration curve.

3.3.1. 3 Determination of Thyroid Stimulating Hormone

3.3.1.3.1. Principle

TSH is measured using an immunoradiometric technique that is referred to as a "sandwich" test. It was employed mouse monoclonal antibodies that were not competitive since they were directed against two separate TSH epitopes. The first monoclonal antibody-coated tubes containing the samples or calibrators are incubated in the presence of the second monoclonal antibody that has been iodine 125 labeled.

Following incubation, the tubes' contents were aspirated, and the tubes were washed to eliminate any unbound 125I_ tagged antibody. Gamma counter analysis was used to determine the bound radioactivity. By extrapolating from the standard curve, the TSH concentrations in the samples were determined. The samples' TSH concentrations were proportionate to the radioactivity (Pierce and Parsons, 1981; Musto et al., 1984; Benkirane et al., 1987).The normal TSH Value = 0.28 –6.82 mlU/ml.

3.3.1.3.2. Reagents

1- Anti-TSH antibody-coated tubes: 2 tubes of 50 each (ready to use).

2-125I-labeled monoclonal anti-TSH antibody: each ready-to-use 11 ml vial contains 515 KBq of 125I-labeled immunoglobulin in a buffer comprising bovine serum, sodium azide (0.1%), and a dye at the time of production.

3- Calibrators: 7 ready-to-use 1 ml vials with a range of 0 to 50 mlU/L of TSH in bovine serum and 0.1 percent sodium azide make up the calibrators.

4- Control serum: two lyophilized vials of TSH that has been frozen in bovine serum are included.

5. One 50 ml bottle of wash solution (20 X).

3.3.1.3.3.procedure

Tubes	Total	Calibrator	Control	Sample
Calibrator		100 µl		
Contro			100µl	
Sample			100 µl	
Tracer	100 µl	100 µl	100 µl	100 µl
Incubated 1 hour at (18 – 25 ˚C) with shaking (>280 rpm).				
Wash solution		2000 µl	2000 µl	2000 µ
The fluid was decanted and blotted on filter paper.				
Wash solution		2000 µl	2000 µl	2000 µ
The fluid was decanted and blotted on filter paper.				
Radioactivity was Counted by gamma counter.				
The results were calculated by calibration curve.				

3.3.2Measurements of Brain and Thyroid Factors

3.3.2.1 Determination of Rat Paired box protein (PAX-8)

3.3.2.1.1 Principle

Enzyme-Linked Immunosorbent Assay is what this kit is for (ELISA). Rat PAX8 antibody has been used to pre-coat the plate. The sample's PAX8 is introduced, and it binds to the antibodies that have been coated on the wells. Then, rat PAX8 antibody that has been biotinylated is added, and it binds to PAX8 in the sample. The biotinylated PAX8 antibody is then bound by the addition of streptavidin-HRP. Unbound Streptavidin-HRP is removed during a washing step after incubation. The amount of Rat PAX8 is then correlated with the development of color in the substrate solution. By adding an acidic stop solution, the process is stopped, and absorbance is then measured at 450 nm.

3.3.2.1.2 Reagent Preparation

1- **Standard** Reconstitute 120 µl of the standard with 120 µl of the standard diluent to make a standard stock solution that has 6.4ng/ml of the standard (12.8ng/ml) in it. Stir the standard slowly for 15 minutes before making dilutions. Using a standard diluent, serially diluted the standard stock solution (6.4ng/ml) to produce solutions with concentrations of 3.2ng/ml, 1.6ng/ml, 0.8ng/ml, and 0.4ng/ml. The standard diluent (0 ng/ml) is used to symbolize the zero standard. Any extra solution needs to be used within a month and frozen at -20°C.

2-**Wash Buffer** :Buffer Wash Buffer 20 ml should be diluted. 25 times of a solution in deionized or distilled water yields 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, gently stir the mixture until the crystals are completely dissolved.

3.3.2.1.3Procedure

1. Before use, prepare all reagents, standard solutions, and samples at room temperature.

2. A standard well was topped out with 50µl of standard solution.

3. The sample was put to the sample wells along with 10 µl of an anti-PAX8 antibody. The sample wells and standard wells then received 50 µl of streptavidin-HRP (Not blank control well). Seal the plate with a sealer and let it sit in the incubator for 60 minutes at 37 °C.

4. The wells were filled with at least 0.35 ml of wash buffer and submerged for 30 to 1 minutes each time the plate was rinsed with wash buffer after the sealer had been removed. Aspirate or decant each well and wash it automatically. Five times with the wash buffer. To blot the plate, put some paper towels or another absorbent item nearby.

5. Substitute cure A 50 µl addition was made to each well, and then 50 µl of substrate solution B was added to each well. The plate was then sealed once more, and it was left to sit at 37 °C without any light for 10 minutes.

6. Dispatch Alternative Each well took in 50 µl, which immediately turned the blue
7. Within 10 minutes of applying the stop solution, calculate the optical density (OD value) of each well using a microplate reader set to 450 nm.

3.3.2.1.4Calculation of Result

Create a standard curve by plotting the average OD for each standard on the Y axis against the concentration on the X axis. Then, construct the best fit curve across the graph's points. To perform these calculations, it is better to use computer-based curve-fitting software. Regression analysis can be used to find the best fit line.

3.3.2.2. Determination of Rat Forkhead box protein E1(FOXE1)

3.3.2.2.1 Principle

Enzyme-Linked Immunosorbent Assay is what this kit is for (ELISA). Rat FOXE1 antibody has been used to pre-coat the plate. When FOXE1 from the sample is added, it binds to the antibodies that have been coated on the wells. Then, rat FOXE1 antibody that has been biotinylated is added, and it binds to FOXE1 in the sample. The biotinylated FOXE1 antibody is then bound by the addition of streptavidin-HRP. Unbound Streptavidin-HRP is removed during a washing step after incubation. After that, a substrate solution is added, and color develops in accordance to the Rat FOXE1 concentration. By adding an acidic stop solution, the process is stopped, and absorbance

3.3.2.2.2 Reagent Preparation

1- **Standard** Reconstitute 120 µl of the standard with 120 µl of the standard diluent to make a standard stock solution that has 6.4ng/ml of the standard (12.8ng/ml) in it. Stir the standard slowly for 15 minutes before making dilutions. Using a standard diluent, serially diluted the standard stock solution (6.4ng/ml) to produce solutions with concentrations of 3.2ng/ml, 1.6ng/ml, 0.8ng/ml, and 0.4ng/ml. The standard diluent (0 ng/ml) is used to symbolize the zero standard. Any extra solution needs to be used within a month and frozen

2-**Wash Buffer** :Wash Buffer 20 ml should be diluted. 25 times of a solution in deionized or distilled water yields 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, gently agitate the mixture until the crystals are completely dissolved.

3.3.2.2.3 Procedure

1. Before use, prepare all reagents, standard solutions, and samples at room temperature.

2. 50 µl of the standard solution were poured into a standard well to fill it up.

3. After adding the sample and 10 µl of an anti-FOXE1 antibody to the sample wells, 50 µl of streptavidin-HRP was added to the sample wells and standard wells (Not blank control well). After sealing the plate with a sealer, incubate for 60 minutes at 37°C.

4. The plate was washed five times with wash buffer by first removing the sealer, then filling the wells with at least 0.35 cc of wash buffer and submerging them for 30 to 1 minute each time. Aspirate or decant each well for automatic washing. Make five uses of the wash buffer. To blot the plate, place paper towels or some other absorbent material close by.

5. Supportive treatment A 50 µl addition was made to each well, and then 50 µl of substrate solution B was added to each well. The plate was then sealed once more, and it was left to sit at 37 °C without any light for 10 minutes.

6. Dispatch Alternative Each well took in 50 l, which immediately turned the blue hue yellow.

7. Within 10 minutes of applying the stop solution, calculate the optical density (OD value) of each well using a microplate reader set to 450 nm.

3.3.2.2.4 Calculation of Result

Create a standard curve by plotting the average OD for each standard on the Y axis against the concentration on the X axis. Then, construct the best fit curve across the graph's points. To perform these calculations, it is preferable to use computer-based curve-fitting software. Regression analysis can be used to find the best fit line.

3.3.2.3. Determination of Rat Homeobox protein (Nkx-2.1)

3.3.2.3.1 Principle

Enzyme-Linked Immunosorbent Assay is what this kit is for (ELISA). Rat NKX2-1 antibody has been pre-coated on the plate. The sample's NKX2-1 is introduced and binds to the antibodies that have been coated on the wells. After that, rat NKX2-1 antibody that has been biotinylated is added, and it binds to NKX2-1 in the sample. The biotinylated NKX2-1 antibody is then bound by the addition of streptavidin-HRP. During a washing stage, unbound Streptavidin-HRP is removed. Following the addition of substrate solution, color changes according to the concentration of rat NKX2-1. By adding an acidic stop solution, the process is stopped, and absorbance is then measured at 450 nm.

3.3.2.3.2 Reagent Preparation

1- **Standard** Reconstitute 120 µl of the standard with 120 µl of the standard diluent to make a standard stock solution that has 6.4ng/ml of the standard (12.8ng/ml) in it. Stir the standard slowly for 15 minutes before making dilutions. Using a standard diluent, serially diluted the standard stock solution (6.4ng/ml) to produce solutions with concentrations of 3.2ng/ml, 1.6ng/ml, 0.8ng/ml, and 0.4ng/ml. The standard diluent (0 ng/ml) is used to symbolize the zero standard. Any extra solution needs to be used within a month and frozen at -20°C.

2-**Wash Buffer** Blend 20 ml of Wash Buffer. By dilution 25 times with deionized or distilled water, 500 ml of 1x Wash Buffer can be created. If crystals have formed in the concentrate, mix it gently until they are completely dissolved.

3.4.2.5.3 Procedure

1. Before use, prepare all reagents, standard solutions, and samples at room temperature.

2. A standard well was topped out with 50µl of standard solution.

3. The sample and 50 µl of streptavidin-HRP were added to the sample wells and standard wells, then 10 µl of an anti-NKX2-1 antibody (Not blank control well). After using a sealer to close the plate, incubate it for 60 minutes at 37 °C.

4. After removing the sealer, the plate was immersed in wash buffer five times, each time for 30 to 1 minute, and the wells were filled with at least 0.35 ml of wash buffer. Aspirate or decant every well before washing it automatically. Put the wash buffer to use five times. Set up some paper towels or other absorbent materials close by to blot the plate.

5. Supportive treatment A 50 µl addition was made to each well, and then 50µl of substrate solution B was added to each well. The plate was then sealed once more, and it was left to sit at 37 °C without any light for 10 minutes.

6. Dispatch Alternative Each well took in 50 µl, which immediately turned the blue hue yellow.

7. Within 10 minutes of applying the stop solution, calculate the optical density (OD value) of each well using a microplate reader set to 450 nm.

3.3.2.3.4 Calculation of Result

Create a standard curve by plotting the average OD for each standard on the Y axis against the concentration on the X axis. Then, construct the best fit curve across the graph's points. To perform these calculations, it is better to use computer-based curve-fitting software. Regression analysis can be used to find the best fit line.

3.3.2.4 Determination of Rat Brain derived neurotrophic facor (BDNF)

3.3.2.4.1 Principle

This kit is for Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with rat BDNF antibody. BDNF from the sample binds to the antibodies that have been coated on the wells when it is added. The biotinylated rat BDNF antibody is then added, and it binds to the sample's ACH BDNF. The addition of streptavidin-HRP then binds the biotinylated BDNF antibody. After incubation, unbound Streptavidin-HRP is eliminated by a washing process. Substrate solution is then added, and color results are used to modify the amount of Rat BDNF. The process is halted by adding an acidic stop solution, and after that, absorbance is measured at 450 nm.

3.3.2.4.2 Reagent Preparation

1- **Standard**: 1. Reconstitute 120 µl of the standard with 120 µl of the standard diluent to make a standard stock solution that contains 12.8 ng/ml of standard but only 6.4 ng/ml of standard. For 15 minutes, carefully mix the standard before making dilutions. The standard stock solution (6.4ng/ml) should be serially diluted by a factor of 2:1 with a standard diluent to produce solutions with concentrations of 3.2ng/ml, 1.6ng/ml, 0.8ng/ml, and 0.4ng/ml. Standard diluent (0 ng/ml) serves as a representation of the zero standard. Any extra solution needs to be frozen at -20°C and used within a month..

2-**Wash Buffer** Wash Buffer 20 ml should be diluted. 25 times of a solution in deionized or distilled water yields 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, gently agitate the mixture until the crystals are completely dissolved.

3.3.2.4.3Procedure

1. Before use, prepare all reagents, standard solutions, and samples at room temperature.

2. A standard well was topped out with 50µl of standard solution.

3. The sample was put to the sample wells along with 10 µl of an anti-BDNF antibody. The sample wells and standard wells then received 50 µl of streptavidin-HRP (Not blank control well). Seal the plate with a sealer and let it sit in the incubator for 60 minutes at 37 °C.

4. The wells were filled with at least 0.35 ml of wash buffer and submerged for 30 to 1 minutes each time the plate was rinsed with wash buffer after the sealer had been removed. Aspirate or decant each well and wash it automatically. Five times with the wash buffer. To blot the plate, put some paper towels or another absorbent item nearby.

5. Substitute cure A 50 µl addition was made to each well, and then 50 µl of substrate solution B was added to each well. The plate was then sealed once more, and it was left to sit at 37 °C without any light for 10 minutes.

6. Dispatch Solution Each well received 50 µl, causing the blue tint to instantly become yellow.

7. The optical density (OD value) of each well should be determined using a microplate reader set to 450 nm within 10 minutes of adding the stop solution.

3.3.2.4.4Calculation of Result

Create a standard curve by plotting the average OD for each standard on the Y axis against the concentration on the X axis. Then, construct the best fit curve across the graph's points. To perform these calculations, it is better to use computer-based curve-fitting software. Regression analysis can be used to find the best fit line.

3.3.2.5 Determination of Rat Acetylcholine (ACH)

3.3.2.5.1 Principle

Enzyme-Linked Immunosorbent Assay is what this kit is for (ELISA). Rat ACH antibody has been used to pre-coat the plate. When ACH from the sample is added, it binds to the antibodies that have been coated on the wells. Then, rat ACH antibody that has been biotinylated is introduced, and it binds to ACH in the sample. The biotinylated ACH antibody is then bound by the addition of streptavidin-HRP. Unbound Streptavidin-HRP is removed during a washing step after incubation. After then, the substrate solution is added, and color develops in direct proportion to the Rat ACH concentration. By adding an acidic stop solution, the process is stopped, and absorbance is then measured at 450 nm.

3.3.2.5.2 Reagent Preparation

1- **Standard** Reconstitute 120µl of the standard with 120 µl of the standard diluent to make a standard stock solution that has 6.4ng/ml of the standard (12.8ng/ml) in it. Stir the standard slowly for 15 minutes before making dilutions. Using a standard diluent, serially diluted the standard stock solution (6.4ng/ml) to produce solutions with concentrations of 3.2ng/ml, 1.6ng/ml, 0.8ng/ml, and 0.4ng/ml. The standard diluent (0 ng/ml) is used to symbolize the zero standard. Any extra solution needs to be used within a month and frozen at -20°C.

2-**Wash Buffer** : Wash Buffer 20 ml should be diluted. 25 times of a solution in deionized or distilled water yields 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, gently agitate the mixture until the crystals are completely dissolved.

3.3.2.5.3Procedure

1. Before use, prepare all reagents, standard solutions, and samples at room temperature.

2. A standard well was topped out with 50µl of standard solution.

3. After adding the sample and 10 µl of an anti-ACh antibody to the sample wells, 50 µl of streptavidin-HRP was added to the sample wells and standard wells (Not blank control well). After sealing the plate with a sealer, incubate for 60 minutes at 37°C.

4. The wells were filled with at least 0.35 ml of wash buffer and submerged for 30 to 1 minutes each time the plate was rinsed with wash buffer after the sealer had been removed. Aspirate or decant each well and wash it automatically. Five times with the wash buffer. To blot the plate, put some paper towels or another absorbent item nearby.

5. Substitute cure A 50 µl addition was made to each well, and then 50 µl of substrate solution B was added to each well. The plate was then sealed once more, and it was left to sit at 37 °C without any light for 10 minutes.

6. Dispatch Solution Each well received 50 µl, causing the blue tint to instantly become yellow.

7. Use a microplate reader set to 450 nm to determine each well's optical density (OD value) within 10 minutes of adding the stop solution.

3.3.2.5.4Calculation of Result

Build the best fit curve across the points on the graph by first plotting the average OD for each standard on the Y axis against the concentration on the X axis. The best curve-fitting software for computers should be used to perform these calculations in order to identify the best fit line using regression analysis.

3.5. Histological study

3.5.1 Histological Preparations

The experience , animals were anesthetized using a ketamine (80 mg/kg) and xylazine (12 mg/kg) cocktail at a dose of 0.1 ml/100 gm body weight (BW) intraperitoneal for isolation of tissue for evaluation histology change study (brain and thyroid gland) were prepared on microscopic slides. As developed samples, putting in a clean container on formalin solution10 was outlined as the following:

1. Fixation : For 48 hours, the samples were placed in a labeled container containing 10% formalin . To ensure that the fluid covered the whole surface and that no particles stuck to the bottom or edges, the container was gently shaken several times (A shank of glass wool placed in the container will aid in keeping the tissue free of the bottom).

2. Dehydration : Increasing amounts of ethanol (50, 60,70,80, 90,100) were used to dehydrate the material (two hours for each concentration).

3. Clearing : The sample was exposed to xylene for 20-25 minutes.

4. Infiltration : The sample was initially infiltrated for 30 minutes at 57–58°C in paraffin-xylene (1:1), and then for 2 hours at 58–60°C in paraffin alone.

5. Embedding : The sample was encased in pure paraffin wax, which hardened at room temperature after melting at a temperature between 58 and 60°C.

6. Sectioning : Using a electronic microtome, the paraffin block was cut into slices with a thickness of 5 microns. The sections were then transferred to a slide coated with Mayer's albumin. The tissue piece was briefly submerged in water that was between 35 and 40 degrees Celsius.

7. Rehydration (Staining) : The slide was first stained with xylene for 15 to 20 minutes, then with ethanol in declining percentages (90, 80, and 70%) for two minutes each, and lastly with distilled water. The slide was then rinsed with distilled water for 5 minutes before being stained with haematoxylin for 10 to 20 minutes. After one minute in acidic alcohol, the slide was rinsed with distilled water. After cleaning, the slide was exposed to eosin stain for 10 to 15 seconds before being immersed in ethanol at increasing concentrations (70, 80, 90, and 99%). (two minutes for each concentration). After 10 minutes, xylene was used to clean the slide.

8. Mounting : A Canada balsam was used to mount the slide, which was then covered with a cover slip. The slide was then scrutinized under a microscope to look for histological alterations.

3.5.2 Examination of Histological Slides and Photography

The tissue sections were examined by light compound microscope (Olympus) with a digital camera (Canon) and then filmed by the microscope with a computer camera.

3.6 Statistical Evaluation Methods:

Statistical tests were conducted using a data were analyzed using SPSS descriptive analysis (Mean-+ S.E) and the signification by using Duncan at P<_ 0.01 (the significance level was (0.01) and the P-value was (0.05), respectively).

4. Results and Discussion

4-1Thyroid Hormones and Thyroid Stimulating Hormone for Mother's .

The results in table (4-1) showed a significant decrease in levels of thyroid-stimulating hormone (TSH) and increase in levels thyroxine (T4) and triiodothyronine (T3) hormones in mother rats with high iodine doses compared with the control group with significant different at ($p \leq 0.01$) as that show in table (4-1) .

Table(4-1): The Effect of Different Doses of Exogenous Iodine on Thyroid Functions of Mother Rat.

	Doses of Iodine(M±SE)					**F-test**	**P-value**
Hormones	**Control**	**0.06 mg/ml**	**1.8 mg/ml**	**5 mg/ml**	**20 mg/ml**		
TSH (mIu/ml)	2.13±0.287 a	1.99 ±0.112 b	1.37 ±0.065 d	1.00 ±0.097 c	0.78 ±0.112 b	263.88	0.0001
T4 (nmol/l)	1.52 ±0.124 a	2.29 ±0.070 b	3.35 ±0.040 c	4.32 ±0.074 d	6.56 ±0.110 e	487.54	0.0001
T3 (nmol/l)	1.58 ±0.041 a	2.3 ±0.065 b	2.65 ±0.287 b	4.27 ±0.066 c	5.29 ±0.076 d	117.02	0.0001

-All results show significant difference at ($P<0.01$) .

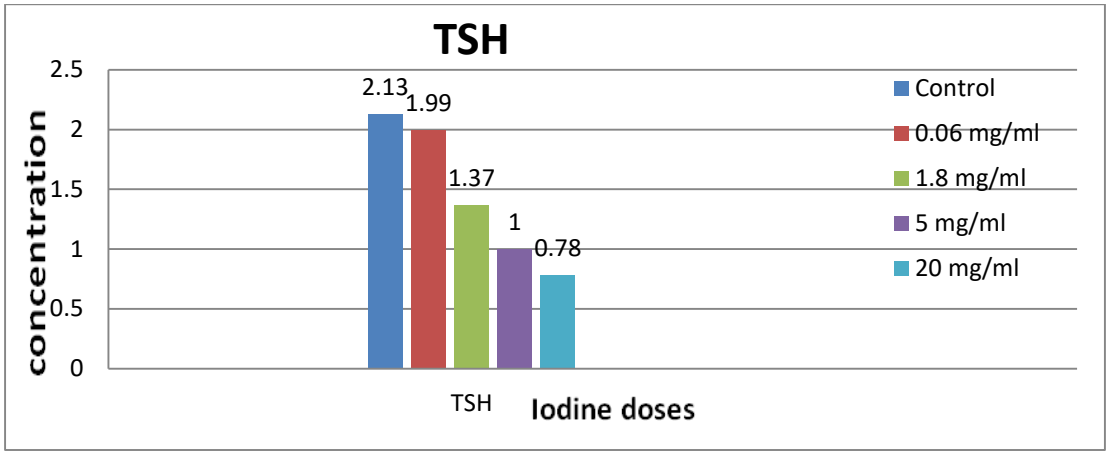


Figure (4-1): The Effect of Exogenous Iodine on Mother's Thyroid-Stimulating Hormone (TSH) .

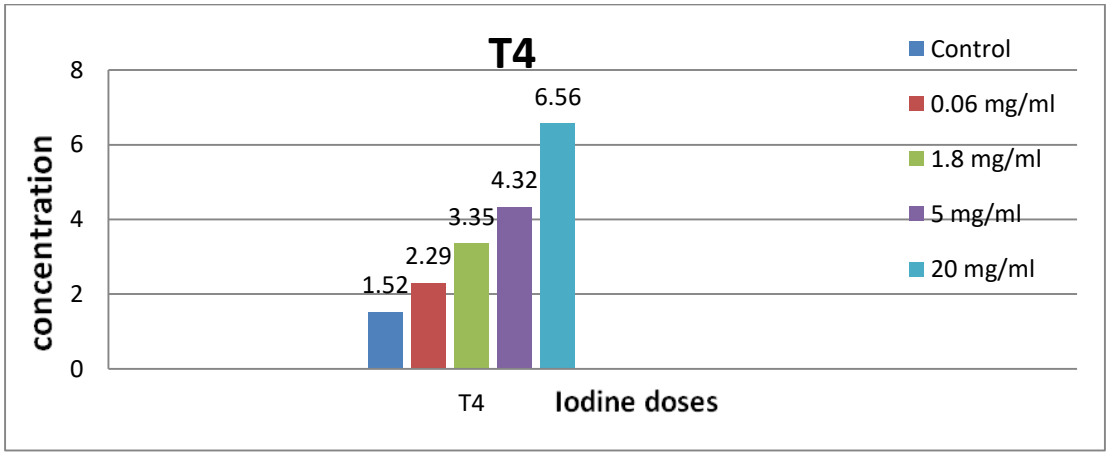


Figure (4-2): The Effect of Exogenous Iodine on Mother's Thyroxine (T4) Hormone .

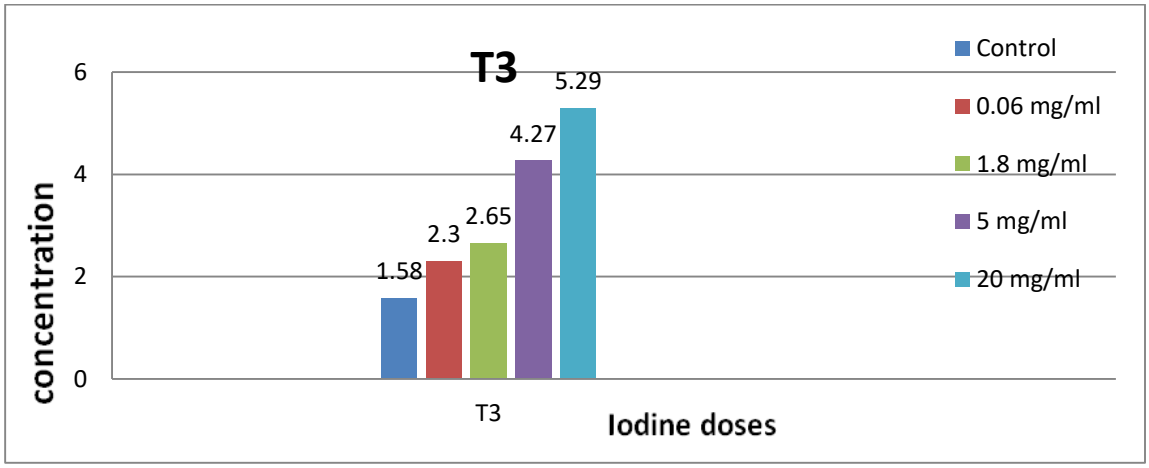


Figure (4-3): The Effect of Exogenous Iodine on Mother's Triiodothyronine (T3) Hormone .

This study in table (4-1) found out that the significant decrease in levels of thyroid-stimulating hormone (TSH) in high iodine doses compared with control group ,and this is a significant increase ($P<0.01$) in mother rats, also the study revealed a significant increase in levels thyroxine (T4) and triiodothyronine (T3) hormones in mother rats at ($P< 0.01$) with high iodine doses compared with the control group, this study agrees with (Sun *et al.*, 2022) .

Results of the present study are in good agreement with study regarding the role of Iodine during pregnancy on thyroid function of mother and fetal in rats, mice, and people exposed to high doses of iodine (Calil-Silveira *et al.*,2016). According to certain research that too much iodine might lead to hyperthyroidism (Roti and Uberti, 2001; Farebrother *et al.*, 2019) .

First , rats need 0.15㎎ of Iodine per gram of food as the minimum amount (National Research Council, 1995) . The results show that these doses of excessive iodine cause a state of hyperthyroidism, who investigated the effects of chronic excess iodine intake on thyroid function and oxidative stress in hyperthyroid rats, they discovered that the groups with or without administration of excess iodine (3000 or 6000 g/L) for 8 weeks had similar results excessive iodine consumption boosted T3 and T4, additionally the high iodine consumption markedly reduced the expression of the NIS, D1 deiodinase, and TPO genes, MDA and antioxidants were markedly elevated in rats with excess iodine in both normal and hyperthyroid conditions as the results corroborated those according to certain research (Hussein *et al.*, 2012).

For thyroid hormones and TSH the results in table (4-1) showed an decrease in TSH level while there were increase in both T3 and T4 levels in mother's pregnancy rats compared with control group, this study agrees with (Rahbani *et al.*,2004; Yu *et al.*, 2006; Kale *et al.*,2007).

Thyroid hormones which produce over activity of sympathetic nervous system, resulting in the increase release of norepinphrine from sympathetic nerve endings in adipose tissue, the fat cells express adrenergic receptors that are stimulated by norepinephrine (Baig *et al.*, 2003) . Thyroid insufficiency leads to generalized slowing of calorigenic metabolism, including over all decreases in daily energy intake, oxygen consumption, and thermogenesis according to research (Scarpace *et al.*, 1997; Bornstein *et al.*, 1997).

Our results explain the effect of the supplemented with Iodine excess during pregnancy and breastfeeding taken significantly inhibited thyroidal D1 activity and mRNA expression significantly increased serum T3 and T4 concentrations and these results supported and agreement with (Wang *et al*., 2009 ; Gao *et al.*, 2013 ; Serrano-Nascimento *et al.*, 2017) .

Similar results were found by established that both the obese strain (OS) with a genetic background predisposed to spontaneous autoimmune thyroiditis and the wild-type were affected by high-dose iodine-induced thyrocyte injury. However, after iodine-induced cell damage, only OS showed significant and persistent lymphocytic infiltration made up of CD4+ T-cells, CD+8 T-cells, B-cells, and macrophages thyrocyte damage and the subsequent lymphocytic infiltration brought on by iodine were both fully avoided by pre-treatment with the antioxidant medication ethoxyquin according to research (Bagchi *et al.*,1995).

According to this study, individuals who consume excessive amounts of iodine may experience thyrocyte damage from oxidative stress; however, whether this cell damage results in lymphocytic infiltration will rely on the additional impacts of genetic variables, iodine treatment greatly enhances intracellular and extracellular reactive oxygen species (ROS) in thyrocytes generated from NOD, according to in vitro studi mouse H-2h4, additionally, it increased ICAM-1 expression, which is essential for steering lymphocytes toward the thyroid, inhibition of nicotinamide adenine dinucleotide phosphate subunit protein

(NADPH) oxidase p67^{phox} (an enzyme that catalyzes the generation of ROS products), iodine-induced ROS generation and ICAM-1 expression in NOD are both eliminated when their inhibitor, diphenyleneiodonium, is used before the iodine therapy, iodine-induced ICAM-1 expression is linked to ROS levels in NOD, according to H-2h4 thyrocytes according the research (Burek and Rose, 2008).

4.2 Thyroid Transcription Factors for Embryo and Mother's .

The results in tables (4-2) and (4-3) showed a significant the levels of thyroid transcription factors (NKX 2.1, FOXE-1 and PAX-8) were decrease in embryo rats while increase in mother's rats with high iodine doses compared with the control group with significant different at ($p \leq 0.01$) as show in tables (4-2) and (4-3) .

Table(4-2): The Effect of Different Doses of Exogenous Iodine on Embryonic Thyroid Transcription Factors .

	Doses of Iodine (M±SE)					**F-test**	**P-value**
Transcription factors	**Control**	**0.06 mg/ml**	**1.8 mg/ml**	**5 mg/ml**	**20 mg/ml**		
FOXE-1 (ng/L)	434.3 ± 19.5 a	290.6 ± 12.9 b	201 ± 23.2 c	159 ± 60.5 d	101 ± 20.4 e	247.73	0.0001
NKX2.1 (ng/L)	3.83 ± 0.193 a	2.27 ± 0.104 b	1.87 ± 0.06 c	1.09 ± 0.172 d	0.91±0.287 e	543.18	0.0001
PAX-8 (ng/L)	427.1 ± 15.1 a	324.49 ±7.61 b	223.05 ±5.20 c	169.3 ±14.8 d	108 ±20.1 e	902.39	0.0001

-All results show significant difference at ($P<0.01$) .

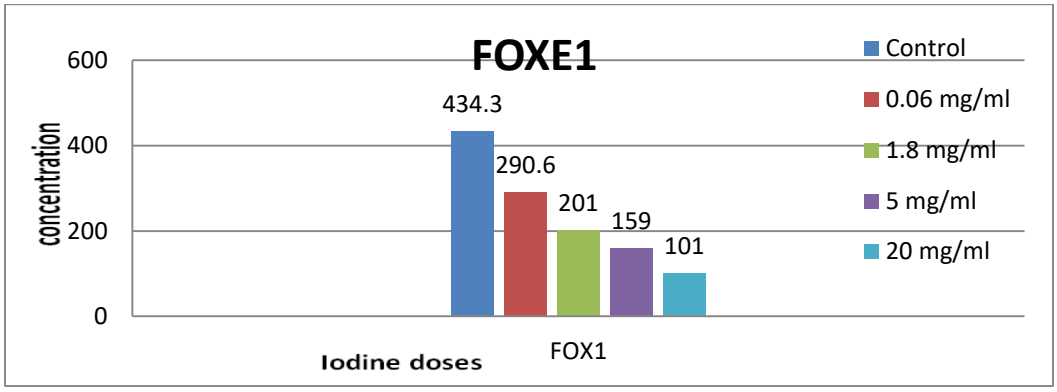


Figure (4-4) : Effect of Exogenous Iodine on The Embryonic Forkhead Box Protein E1(FOXE1) .

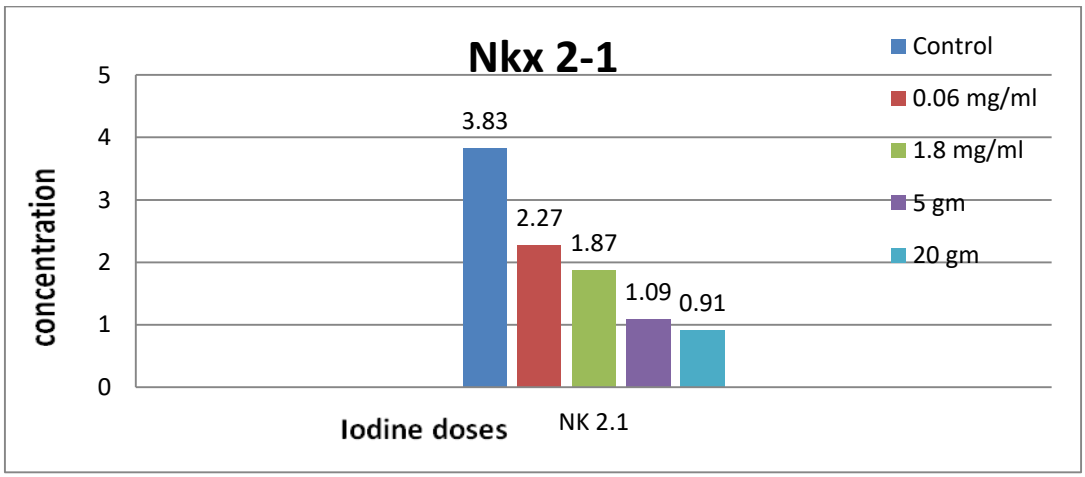


Figure (4-5) : Effect of Exogenous Iodine on The Embryonic Homeo Box Protein (Nkx 2-1) .

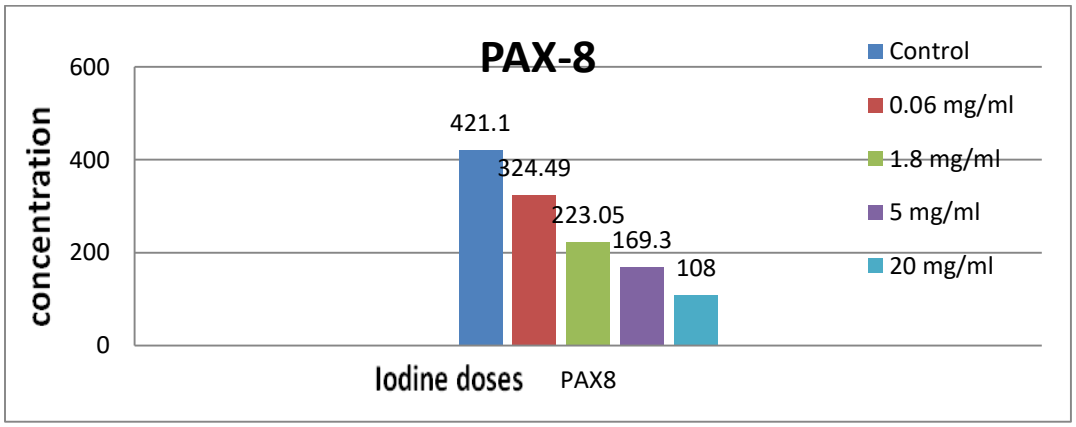


Figure (4-6) : Effect of Exogenous Iodine on The Embryonic Paired Box Gene 8 (PAX-8) .

Table (4-3): The Effect of Different Doses of Exogenous Iodine on Mother Thyroid Transcription Factors .

	Doses of Iodine(M±SE)					**F-test**	**P-value**
Transcription factors	**Control**	**0.06 mg/ml**	**1.8 mg/ml**	**5 mg/ml**	**20 mg/ml**		
FOXE-1 (ng/L)	130 ±16.4 e	170 ± 50.5 d	270.9 ± 53.2 c	490.6 ± 56.9 b	555.3 ± 25.5 a	237.73	0.0001
NKX2.1 (ng/L)	1.1 ± 0.287 e	2.09 ± 0.272 d	3.87 ± 2.33 c	4.27 ± 9.104 b	5.83 ± 3.166	643.18	0.0001
PAX-8 (ng/L)	188 ± 20.1 e	269.3 ± 13.8 d	323.05 ±5.20 c	414.49 ±9.71 b	527.1 ± 27.1 a	912.39	0.0001

-All results show significant difference at (P<0.01) .

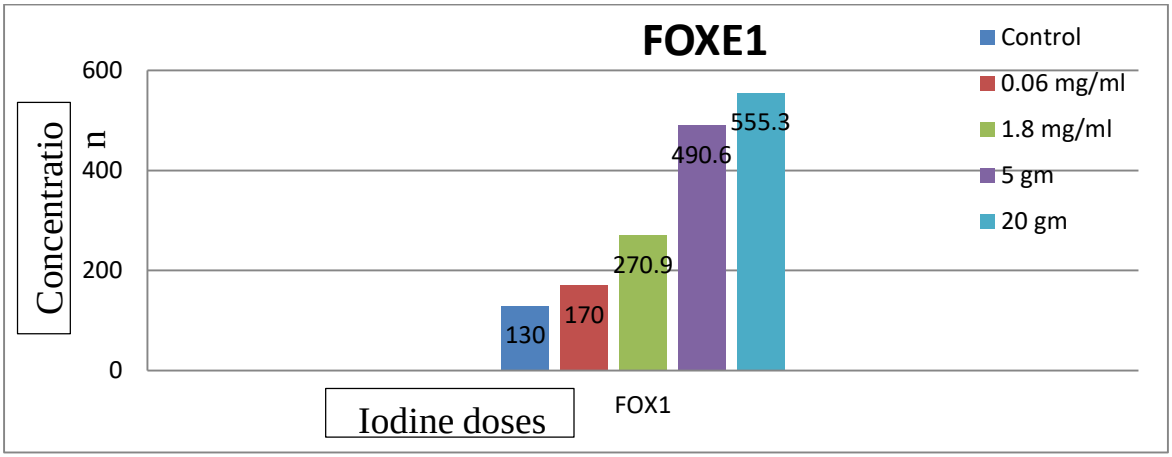


Figure (4-7) : Effect of Exogenous Iodine on The Mother's Forkhead Box Protein E1(FOXE1) .

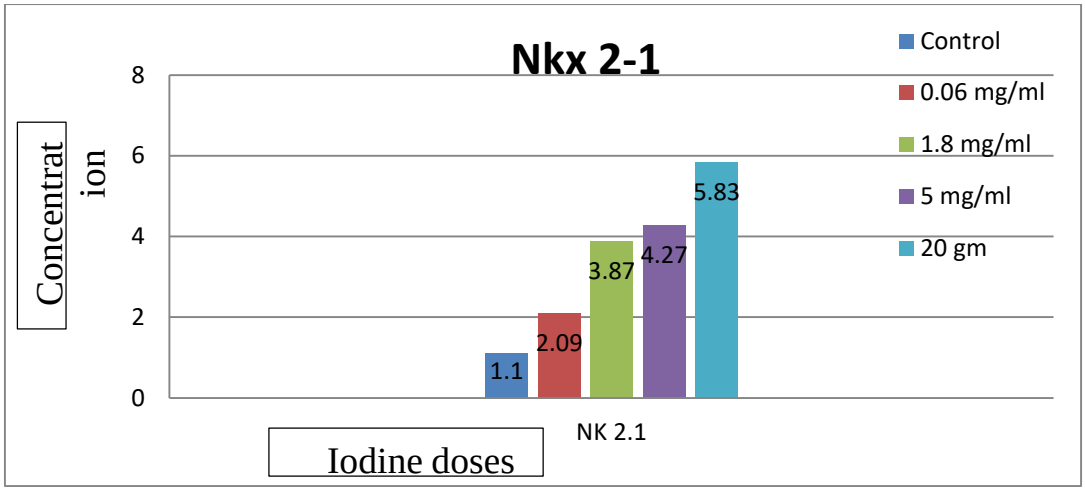


Figure (4-8) : Effect of Exogenous Iodine on The Mother's Homeo Box Protein (Nkx 2-1) .

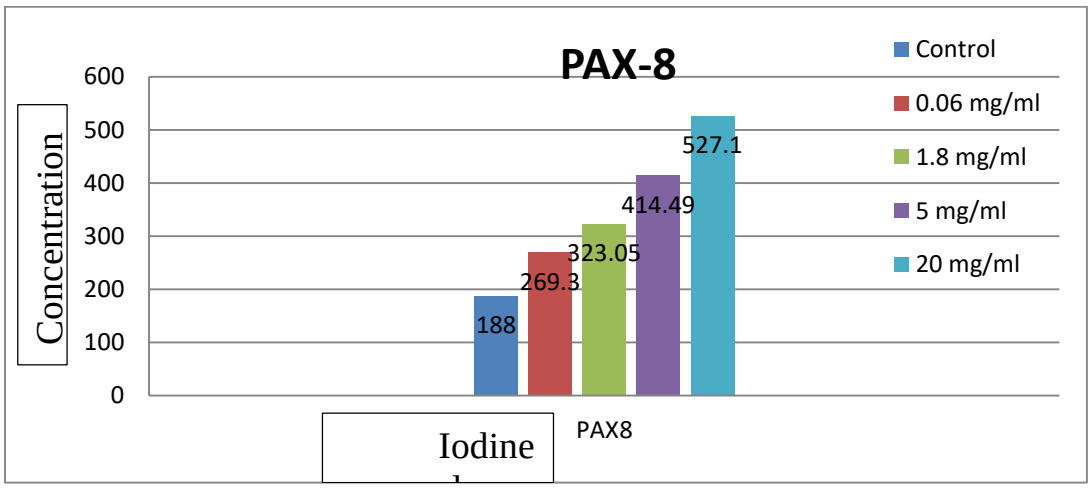


Figure (4-9) : Effect of Exogenous Iodine on The Mother's Paired Box Gene 8 (PAX-8) .

This study in tables (4-2) and (4-3) found out that the significant decrease in levels of thyroid transcription factors (NKX 2-1 FOXE-1 and PAX-8) in embryo rats with high iodine doses compared with control group , and there are a significant decrease ($P<0.01$) in embryo rats , also the study revealed a significant increase thyroid transcription factors mother rats with high iodine doses

compared with the control group with significant different compared with control group ,and there are a significant increase ($P<0.01$) in mother rats , this study agreement with (Zhao *et al.,* 2000) .

Results of the present study are in good agreement with study regarding the role of Iodine on thyroid transcription factors of mother and fetal special high iodine intake during pregnancy when failure to adapt to a high iodine intake, however results in a number of illnesses for mothers a chronically high iodine intake effect directly on high thyroid transcription factors and cause goiter, hasten the progression of subclinical thyroid conditions into overt hypothyroidism or hyperthyroidism, increase the prevalence of autoimmune thyroiditis , and raise the risk of thyroid cancer and lead to low embryo thyroid transcription factor that cause not occur thyrogenesis for embryo result supported by other study that demonstrated an independent (Farebrother *et al*, .2019).

Our results corroborated the papers described, because it is well known how FOXE-1 functions during development and differentiation that agree with (Fernandez *et al.,* 2015; Lopez-Marquez *et al.,* 2019). However, nothing is known about its possible contribution to thyroid carcinogenesis or the associations between the risk of thyroid cancer and various allelic variations in or close to FOXE1and the levels of defined FOXE1 expression are either unaffected or elevated in human malignancies, indicating that it may play a role in the development and spread of these cancers so our results supported and agreement with (Sugimachi *et al.,* 2016).

Thyroid cancer frequently involves the loss of differentiation markers and thyroid-specific proteins , indeed multiple investigations have shown that some thyroid carcinomas express thyroid-specific transcription factors abnormally, and they hypothesize that their deregulation is a crucial step in the development of thyroid neoplasms according to research(Zhang *et al.,* 2006).

Found that the expression of FOXE1 was markedly elevated in Papillary thyroid carcinoma PTC tumor tissues and linked with clinical outcome as a result FOXE1 represents both a new therapeutic target and a possible biomarker for PTC prognosis , additionally it was accepted that thyroid cancer cell lines had increased FOXE1 expression which was discovered by (Fan *et al.,* 2013 ; Morillo-Bernal *et al.*, 2020).

For the first time, it was revealed that the PAX8-PPAR protein tyrosine phosphatase expression was significantly higher in thyroid cancer cell lines and thyroid cancer tissues compared to normal thyroid cell lines and normal and paraneoplastic thyroid tissues, this high expression of PAX8-PPAR may be involved in the development of thyroid cancer and is closely related, the results also showed that PAX-8 was higher, according to research (Fan *et al.*,2022).

Increasing data reveals that ROS-dependent mechanisms control Na+ / I Symporter (NIS) production and function in cancer and non-cancer situations, such as after iodide overload, the Wolff-Chaikoff effect, a mechanism of thyroid auto regulation, is induced by high iodide levels and causes a brief decrease of thyroid hormone production according to research (Eng *et al.,* 1999). Reduced NIS iodide absorption and enhanced apical iodide efflux are thought to be responsible for thyroid escape from the iodide inhibitory effect, which lowers intracellular iodide concentrations and relieves thyroid function inhibition (Calil-Silveira *et al.,* 2016). According to dynamic variations in the cellular redox state are linked to NIS-related responses to iodide excess similar results were found by (Yao *et al*., 2012) . In *vitro* and in *vivo*, NIS mRNA, protein, and activity all decreased as a result of I overload, according to research by (Leoni *et al.,* 2011).

The restoration of the NIS following I therapy required a compensatory rise in thioredoxin reductase antioxidant activity, demonstrating that ROS levels are directly related to the control of the NIS ,accordingly later investigations discovered an acute NIS mRNA drop that was dependent on ROS and NIS

inactivation at the plasma membrane, both of which were prevented by ROS scavengers according to research (Arriagada *et al.,* 2015).

An essential regulator of NIS transcription, PAX-8 is a transcriptional factor necessary for thyroid growth and differentiation. In both human and rat thyrocytes, PAX-8 binds to the NIS upstream enhancer (NUE) and promotes the production of NIS (Taki *et al.*, 2002).

Similar to other PAX family members, the redox state of two cysteine residues—Cys-45 and Cys-57—in its structure determines the PAX8 DNA binding activity, APE1 and a reduction cascade involving thioredoxin reductase-1 (TxnRd1) are required for PAX8 to be decreased in thyroid cells in order for it to bind to NUE and induce the transcription of NIS according to research (Leoni *et al.,* 2016).

Propath Laboratory (2001) numerous investigation has demonstrated that NKX2-1 (TTF-1) antibody reagents can be quite helpful tools in diagnostic pathology it is crucial to understand that only nuclear antibody to TTF-1 indicates true reactivity, and any cytoplasmic reactivity should be disregarded for diagnostic purposes much like with some other nuclear antigens like ER and PR believe .

Thyroglobulin (Tg), thyroid peroxidase (TPO), sodium-iodide symporter (NIS), and thyroid stimulating hormone receptor (TSH-R) gene expression are all regulated by TTF-1 in the adult thyroid gland, given that TSH controls thyroid development and function, there may be some interaction between NKX2-1 (TTF-1) and TSH, according to theory, TSH-R activity can increase TTF-1, which manifests itself in numerous ways in benign and malignant thyroid conditions. In papillary carcinomas, TTF-1 mRNA is usually present, whereas it is lacking in anaplastic carcinomas according to research (Fenton *et al.*,2001).

4.3 Brain Neurotransmitter Parameters for Embryo and Mother's.

The results in tables (4-4) and (4-5) showed a significant the levels of brain neurotransmitter Parameters (Ach and BDNF) increase in embryo and mother rats with high iodine doses compared with the control group with significant different at ($p \leq 0.01$) as show in tables (4-4) and (4-5).

Table (4-4): The Effect of Different Doses of Exogenous Iodine on Embryonic Brain Parameters.

	Doses of Iodine(M±SE)					**Statistical outcomes**	
Brain parameters	**Control**	**0.06 mg/ml**	**1.8 mg/ml**	**5 mg/ml**	**20 mg/ml**	**F-test**	**P-value**
Ach (ng/L)	36.96±0.756 a	50.5 ±1.03 b	65.38 ±1.02 c	88.23 ±1.10 d	135.23 ±0.765 e	1657.39	0.0001
BDNF (ng/L)	1.01±0.0526 a	1.96±0.0581 b	3.67±0.0883 b	9.5 ±0.371 c	21.79 ±0.454 d	1032.83	0.0001

-All results show significant difference at ($P<0.01$) .

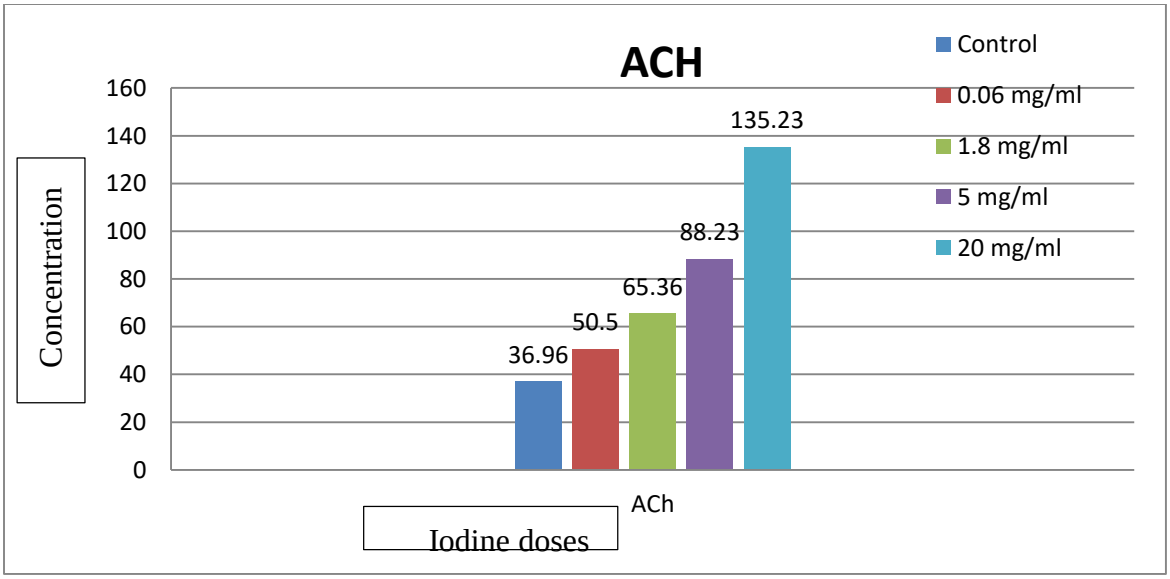


Figure (4-10) : Effect of Exogenous Iodine on the Embryonic Acetylcholine (ACH) .

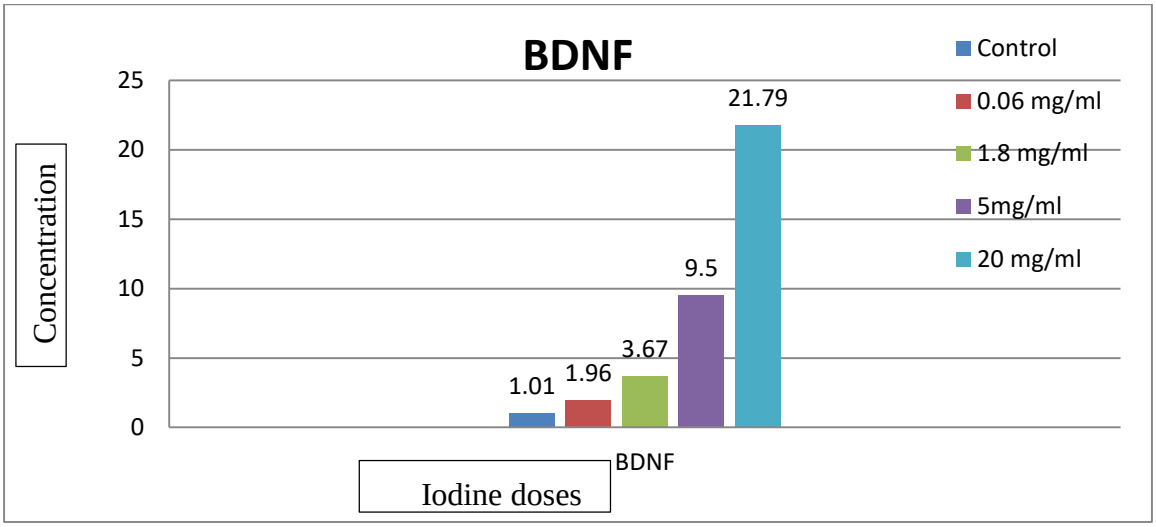


Figure (4-11) : Effect of Exogenous Iodine on the Embryonic Brain-Derived Neurotrophic Factor (BDNF).

Table (4-5): The Effect of Different Doses of Exogenous Iodine on Mother Brain Parameters.

	Doses of Iodine(M±SE)					**Statistical outcomes**	
Brain parameters	**Control**	**0.06 mg/ml**	**1.8 mg/ml**	**5 mg/ml**	**20 mg/ml**	**F-test**	**P-value**
Ach (ng/L)	50.86±0.36 a	66.5±1.03 b	86.18±1.02 c	100.1±1.10 d	150.13±0.765 e	1657.39	0.0001
BDNF (ng/L)	2.11±1.05 a	3.86±0.15 a	5.77±1.33 b	1 0.2±2.30 c	25.81[d]±0.12 d	1032.83	0.0001

-All results show significant difference at (P<0.01) .

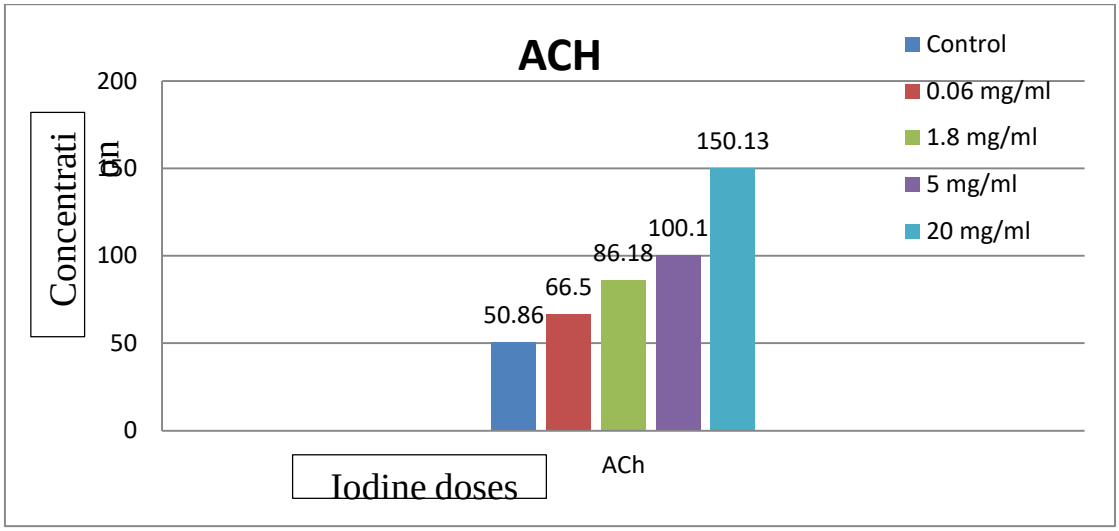


Figure (4-12) : Effect of Exogenous Iodine on the Mother's Acetylcholine (ACH) .

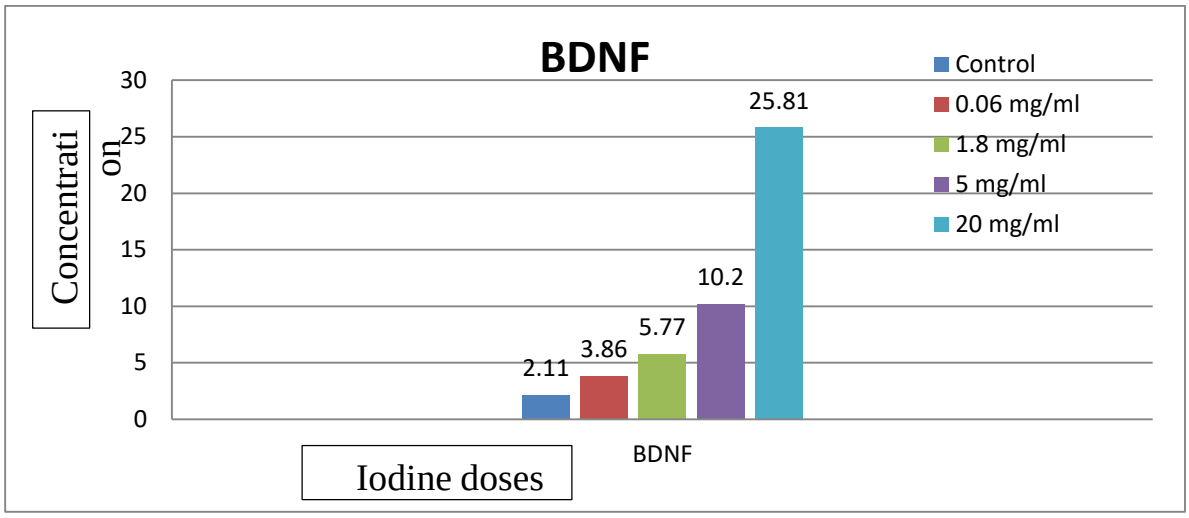


Figure (4-13) : Effect of Exogenous Iodine on the Mother's Brain-Derived Neurotrophic Factor (BDNF) **.**

This study in tables (4-4) and (4-5) found out that the significant increase in levels of brain neurotransmitter Parameters (Ach and BDNF) in embryo and mother's rats with high iodine doses compared with the control group with significant different at ($p \leq 0.01$), this result supported and was in agreement with a study by (Jagadis *et al.*, 2016) .

Our results were found increase in levels of brain neurotransmitter Parameters (Ach and BDNF) that mean lowering the level of Ach in the synaptic cleft, an increase in Ach activity brought on by too much iodine may lower the effectiveness of clolinergic neurotransmission ,so the increased synaptosomal membrane sphingomyelin promotes increasing cognitive impairment caused by decreased Ach levels in the synaptic cleft, particularly in young adults by encouraging or preventing cell death, an elevated Ach level contributes to apoptosis, our results supported and agreement with (Jiang *et al.,* 2008).

Results of the present study are in good agreement with study regarding that when too much iodine is supplemented special during pregnancy abiochemical condition known as hypothyroidism occurs for fetal , which in turn causes a considerable rise in acetylcholine sterase activity in the cerebral cortex and hippocampus but not as much in the hypothalamus and cerebellum according to this study (Jagadis *et al.*,2016)

Our findings supported by increase in the physiological dose of iodine in rat would affect brain development of their offspring female, there is evidence that maternal thyroid hormones can cross the human placenta and act to modulate fetal development before the onset of the fetus's own thyroid hormone production we agree with (James *et al.*,2007) . Recent research have suggested that too much thyroid hormone may hinder fetal brain development and have an impact on the neurological health of newborn rats (Ahmed *et al.*,2010; Chen *et al.*,2011; Jiang *et al.*,2012).

Results of the present study are in good agreement with study regarding the role of Iodine and that effect on neurogenesis human studies that show ills thyroidism affects BDNF expressionstudies have demonstrated that antidepressant treatment, particularly with selective serotonin reuptake inhibitors (SSRIs) combined hormone replacement therapy, improves symptoms like depression and lethargy that are frequently experienced by hypothyroid patients

(Binder, 2004). It has been demonstrated that thyroid hormones(TH) controls 5-hydroxytrptamine (5HT) neurotransmission that latter reduces thyroid regulation hormone (TRH) , TH cause increasing 5HT metabolism , 5HT receptor expression and alters the expression of BDNF and 5HT in the brain according to this study (Madhusudhan *et al.*,2022).

4.4 The Body Weight For Embryo and Mother's

The results in table (4-6) showed a significant the levels of body weight decrease in embryo and mother rats with high iodine doses compared with the control group with significant different at ($p \leq 0.01$) as show in table (4-6).

Table (4-6): The Effect of Different Exogenous Iodine on the Body Weight of Mother and an Embryo.

	Doses of Idoline(M±SE)					**F-test**	**P-value**
weight	**Control**	**0.06 mg/ml**	**1.8 mg/ml**	**5 mg/ml**	**20 mg/ml**		
Body weight of embryo	8.3 ±0.0907 a	6.47 ±0.125 b	5.6 ±0.133 c	4.36 ±0.0991 d	3.3 ±0.109 e	294.35	0.0001
Body weight of mother	288 ±1.03 a	270.5 ±1.92 b	235.2 ±1.74 c	209.5 ±0.980 d	177.8 ±1.58 e	887.39	0.0001

-All results show significant difference at ($P<0.01$)

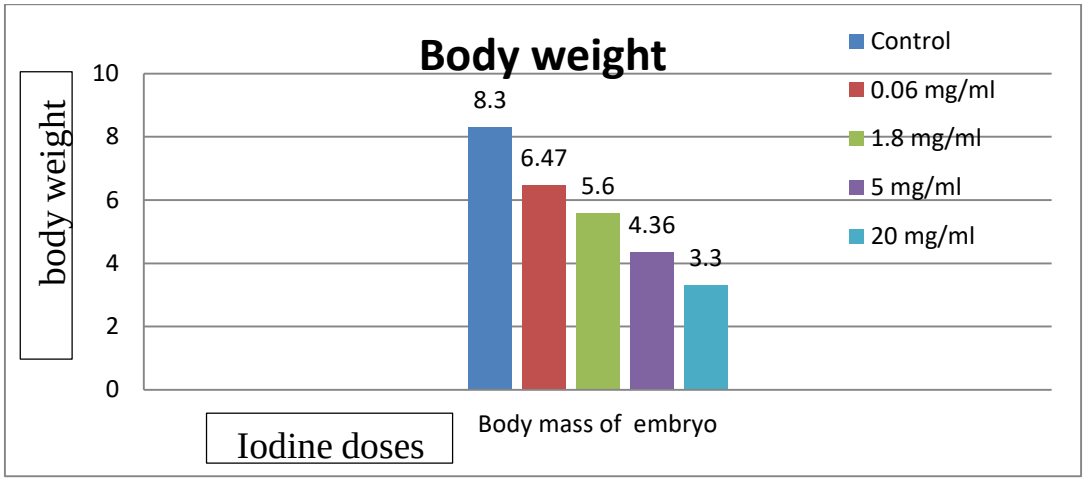


Figure (4-14): The Effect of Exogenous Iodine on the Body Weight of Embryo.

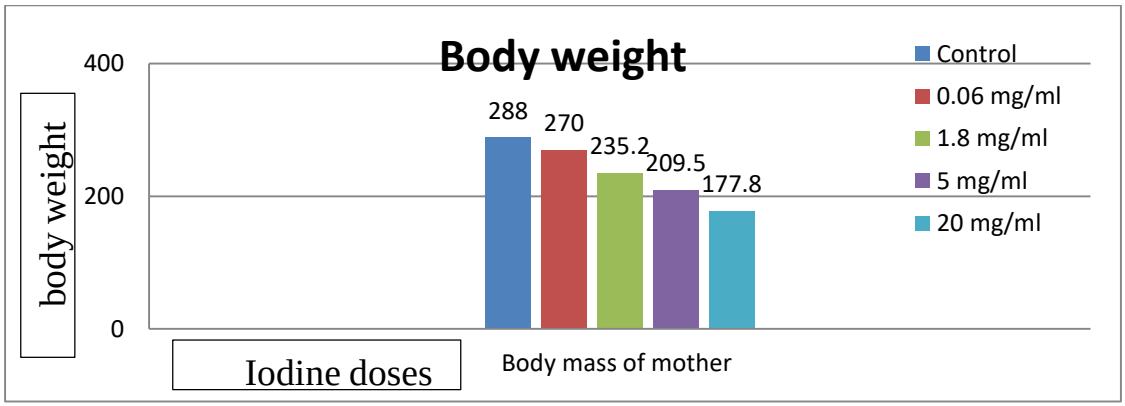


Figure (4-15) : The Effect of Exogenous Iodine on the Body Weight of Mother.

This study in table (4-6) found out that the significant decrease in levels the body weight in embryo and mother's rats with high iodine doses compared with the control group with significant different at ($p \leq 0.01$), our results supported and agreement with a study by (Roti and Uberti , 2001).

These findings were in agreement with those of (Serrano-Nascimento *et al.*, 2017), who utilized iodine during pregnancy and lactation, and our results study supported by (Pickard *et al.*, 2003) .

We were found associated with transient reductions in fetal and mother body weights prior to the onset of fetal hypothyroidism because increasing THs for mother's that raise metabolism , carbohydrolysis, lipolysis and catabolism of protein , these results concur with past research with a study by (Morreale de Escobar *et al.*,1985, Pickard *et al*., 2003).

Our findings show using IE while pregnant or nursing increases the risk of the mother developing thyroid disease and since ID and IE both significantly affect maternal thyroid function, they should be closely monitored , our results agreement with a study by (Liu *et al.,* 2015 ;Alexander *et al.*,2017).

4.5 The Correlation Between Embryonic Parameter .

The findings recorded a positive correlation between the studied parameters as show in table (4-7) .

Table (4-7): The Correlation Matrix Among Studied Embryonic Biomarkers.

parameter	**ACh**	**BDNF**	**FOXE1**	**Nkx-2.1**
ACh		R=0.97, P=0.0001		
FOXE1	R=0.962, p=0.0001	R=0.938, p=0.0001		
Nkx-2.1	R=0.984, p=0.0001	R=0.959, P=0.0001	R=0.952, P=0.0001	
PAX-8	R=0.983, p=0.0001	R=0.951, P=0.0001	R=0.964, P=0.0001	R=0.985, P=0.0001
Body weight of embryo	R=-0.916, P=0.0001	R=-0.853, P=0.0001	R=-0.92, P=0.0001	R=-0.938, P=0.0001

Suggested that there is a connection and cross-communication between the thyroid transcription factors (TTFs),It is undeniably true that all TTFs are expressed in thyroid precursors, but it is debatable if this means that all TTFs are required for the differentiation of a precursor cell into a differentiated thyroid cell, where proven in murine ESC, Nkx2-1 (TTF-1) and Pax8 expression are both necessary to drive differentiation to a thyroid lineage, and Nkx2-1 can also cause Foxe1 expression and that shows that Foxe1 is downstream in the regulatory network along with the finding that Pax8 regulates Foxe1 in mice according to research (Dame *et al.*,2017).

It is believed that Foxe1, which is regarded as a pioneer factor with the innate ability to modify chromatin, directs the thyroid differentiation program, which is in charge of starting the expression of thyroid differentiation genes , a feed forward transcriptional loop may be active during thyroid development because we have discovered an auto-regulatory mechanism for Nkx2-1 and Pax8 expression in thyroid cells in culture according to research (Parlato *et al.*,2004) .

These findings in table (4-7) were in agreement with those of findings point to a hierarchical network among the TTFs and a connection between them and the relationship between the thyroid transcription factors and the genes involved in thyroid differentiation, including how they interact with the newly involved transcription factors, Taz and Hoxb3 control Nkx2-1, which in turn encourages the expression of the other three TTFs, Pax8 is positively regulated by TSH and Taz and controls the expression of Hhex and Foxe1, TGFb also has a detrimental influence on it, TSH and IGF1 both positively regulate Foxe1, the expression of the thyroid differentiation genes Nis (Slc5a5), Tg, and Tpo is regulated by all TTFs agreement with a study by (Lopez-Ma'rquez *et al.*, 2021).

BDNF is a well-known neurotrophic factor that binds to the TrkB receptor on the surface of neuronal cells and has been demonstrated to interact with the signaling pathway, and the results also revealed a positive link between BDNF and Acetylecholine, knockout rats for any neurotrophins or their receptors suffered severe neurological disorders in experimental experiments. Furthermore, it has been demonstrated that the BDNF signaling pathway is essential for neuronal development, survival, plasticity, and cognition (Guo *et al.*,2014) .

When rats experience extreme stress, BDNF expression in the hippocampus of the NRSDG dramatically increased compared to that of the NRSG, demonstrating that -GPC significantly contributes to neurogenesis in the hippocampus after severe stress exposure according to research (Sawamoto *et al.*, 2017 ;Yu *et al.*,2020).

When people or mice are under modest stress or focused on learning something new, the neurotransmitter acetylcholine is released by nerve cells in the brain, a quick-acting enzyme called acetylcholine sterase (ACh) swiftly breaks down the neurotransmitter to stop the nerves from firing too much according to recent research (Mineur *et al.*,2022) .

The study results in table (4-7) found a correlation between BDNF and FoxO1 which agreed with (Liu *et al.*,2020). The regulatory relation of BDNF and FoxO1 in postpartum female mice, we conducted a correlation analysis between BDNF and FoxO1 levels in the mPFC of postpartum female mice that had received CUS treatment showed that total BDNF mRNA levels were substantially linked with FoxO1 at the protein ($P = 0.018$) and mRNA ($P = 0.010$) levels , in PPD female mice, there is a link between BDNF and FoxO1, suggesting that FoxO1 may be a functional target gene for BDNF, the BDNF typically controls the transcriptional activity of FoxO genes by activating TrkB and downstream kinases and in PC12 cells, BDNF had no impact on the

phosphorylation or translocation of the FoxO1 protein , our results supported and agreement with a study by (Gan *et al.*,2005 ; Wen, *et al.*, 2012).

It suggests that the level of protein modification may not be a factor in how BDNF regulates FoxO1 function, Our findings showed in embryo that a particular BDNF lower or deletion can be effect to lower FoxO1 expression, demonstrating that BDNF can influence FoxO1 activity via regulating gene transcription and translation, underlying mechanism, however, appears to be complex because BDNF can activate several signaling pathways, including PI3K/AKT, MEK/ERK, PLC-PKC and PLC-CaMKII, to control certain transcription factors, including CREB and NF-B according to research (Numakawa *et al.*, 2010).

TTF-1 and other thyroid transcription factors, such as PAX8, FOXE1, and HHEX, are coexpressed in thyroid precursor cells and thyroid follicular cells during thyroid development, and their encoding genes are continuously expressed in mature thyroid cells, this helps to establish and maintain the thyroid's phenotype, function, homeostasis, and tissue differentiation, however the thyroid precursor cells undergo apoptosis and vanish in the early stages of embryonic development (E10.5–11.5), Our results of search found the absence or lower of TTFs special in the early stages of embryonic, resulting in thyroid degeneration, a reduction in thyroid follicular density, and aberrant formation of mature thyroid tissue ,our results supported and agreement with a study by (Kusakabe *et al.*, 2006).

The development of interneurons in the ventricular zone is also regulated by TTF-1, which is expressed in the pituitary, hypothalamus, and other diencephalic tissues, also discovered that TTF-1 could control the permissive chromatin state and transcriptional activation in the subventricular and mantle zones, TTF-1 is essential for the generation of TTF-1-derived cell lineages, such

as GABAergic neurons, NG2 glia (or oligodendrocytes), and astrocytes (Sandberg *et al.,* 2016). These findings in table (4-7) were reported a correlation between thyroid factors and brain neurotransmitters biomarkers in our stud these cell lineages include NG2 glia (or oligodendrocytes), NG2 glia or oligoden our results supported and agreement with a study by (Minocha *et al.,* 2015) .

The main factor that promotes the differentiation, development, and proliferation of the thyroid during the embryonic stage is lastly the effect of the thyroid transcription factors and the body weight, which is represented by TTF-1, this effect is also in charge of maintaining the thyroid's normal function after birth , the TTF-1 and other thyroid transcription factors (PAX8, FOXE1, and HHEX) are coexpressed in thyroid precursor cells and thyroid follicular cells during the development of the thyroid, and the genes that encode these transcription factors are continuously expressed in mature thyroid cells (Kusakabe *et al.,* 2008). Congenital hypothyroidism or hyperthyroidism (CH), a common endocrine and metabolic disease in the neonatal period, is a physical and intellectual developmental disorder brought on by a low according to recent research (Guan *et al.*,2021).

Thyroid hormones control numerous metabolic processes and are crucial for growth and development, Thyroglobulin is converted into thyroid hormones in the thyroid gland, where they are produced and secreted in response to thyroid-stimulating hormone (TSH), which is a component of the hypothalamus-pituitary-thyroid axis, which controls the amount of T3 that is readily available in the tissues (van der Spek *et al.,* 2017). Thyroid hormones(THs) are crucial for controlling body weight since they primarily control how much energy is expended (Mullur *et al.*,2014) . Our results of search in tables (4-6 and 4-7) found relationship between thyroid malfunction and major changes in body weight by controlling the THs on resting metabolic rate (RMR), including

hyperthyroidism and hypothyroidism, is well documented , our results supported and agreement with a study by (Liu *et al*.,2017).

4.6 Effect of Iodine on the Reproductive Performance.

The results in tables (4-8) showed a significant the levels of decrease in number of birth with high iodine doses compared with the control group with significant different at ($p \leq 0.01$) as show in table (4-8).

Table (4-8) : The Effect of Different of Exogenous Iodine on the Reproductive Performance Measured by Number of Birth.

	Number of Birth		
Doses of Iodine	**Mean ± SE**	**F- test**	**P-value**
Control	8.3 ±0.153 a	8.27	0.0001
0.06 mg/ml	5.7 ±0.883 ab		
1.8 mg/ml	4.7 ±0.844 b		
5 mg/ml	3.2 ± 0.611 b		
20 mg/ml	2.2 ± 0.629 b		

-All results show significant difference at ($P<0.01$)

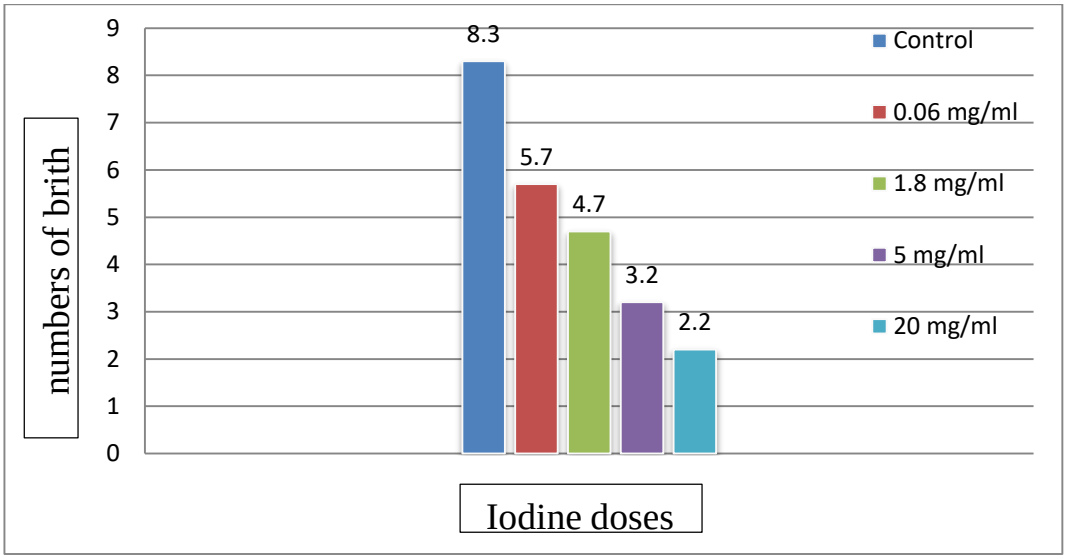


Figure (4-16) : The Effect of Exogenous Iodine on the Reproductive Performance Measured by Number of Birth.

This study in table (4-8) found out that the significant decrease in levels of number of birth with high iodine doses compared with the control group with significant different at ($p \leq 0.01$), this result supported and was in agreement with a study by (Liu *et al.*, 2018) .

These findings corroborated with findings from other studies indicating hypo- and hyperthyroidism decreases the frequency and amplitude of spontaneous rhythmic contractions in the uterus and puberty was markedly delayed of virgin rats agreement with a study by (Parija *et al*., 2001).

These findings show thyroid diseases had reduced endometrial and myometrial volumes and uterine weight that raise levels of abortions or less chance of pregnancy as a result agreement with a study by (Parija, *et al.*, 2006).

Results of the present study are in good agreement with study regarding the role of Iodine and that effect on levels of thyroid hormones(TH) that mean maternal hyperthyroidism and TH dysregulation affects the profiles of reproductive hormones especially estrous cycle also estrogen and progesterone stimulated liver for product thyroglobulin binding proteins, and reduces capacity to ovulate and form corpora lute by reducing the population of resting oocytes, the number of activated follicles, and the primordial follicle pool, also TH effect on human chorionic gonadotropin hormone (HCG) , it stands to reason that maternal hyperthyroidism would be linked to lower fertility rates our results supported and agreement with a study by (Bagheripuor *et al.,* 2018).

4-7 Histological Examination of Embryo and Mother's Thyroid Gland .

The microscopic Histological examine In control groups for thyroid mother's and embryo showed normal histological appearance normal size follicles lined by cuboidal follicular cells as show in (figure: 4-17 & 4-18) ,but the treated groups for thyroid mother's and embryo with (0.06 mg/ml) showing small size thyroid follicles and no colloid production for embryo and variable small size thyroid follicles filled with colloid for mother's as show in (figure: 4-19 and 4-20).

Also in groups treated with (1.8 mg/ml) showed variable small size atrophic thyroid follicles with no colloid production in embryo thyroid while large size thyroid follicles with colloid production for mother thyroid as show in (figure: 4-21 & 4-22), but in groups treated with (5 & 20 mg/ml) reveal very small size atrophic thyroid follicles and no colloid production in embryo thyroid while variable large size follicles lined by active columnar follicular cells with excessive colloid production with scalloping of colloid and follicular cell proliferation for mother's thyroid as show in (figure : 4-23,4-24,4-25 and 4-26) .

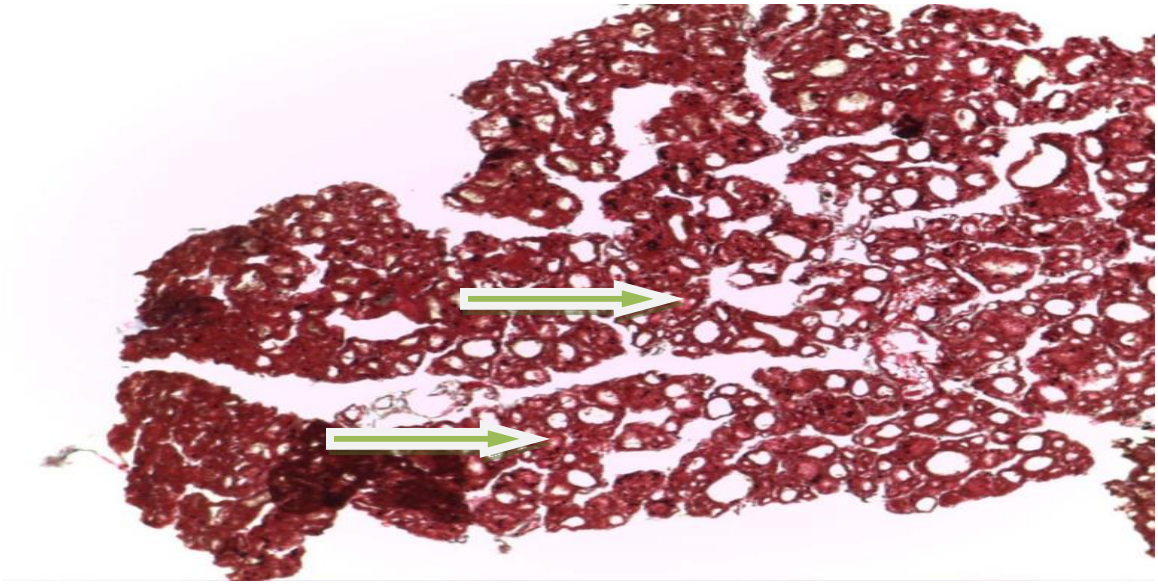


Figure (4-17) : Tissue Section for Control Thyroid Embryo Show: normal size follicles lined by cuboidal follicular cells (H & E 40 / X) .

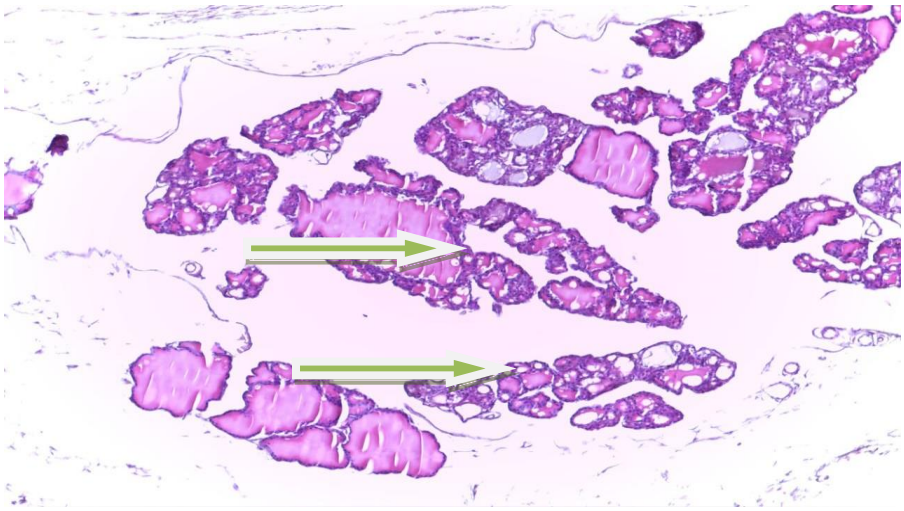


Figure (4-18): Tissue Section for Control Thyroid Mother's Show: normal size normal size follicles lined by cuboidal follicular cells filled with colloid (H & E 400 / X).

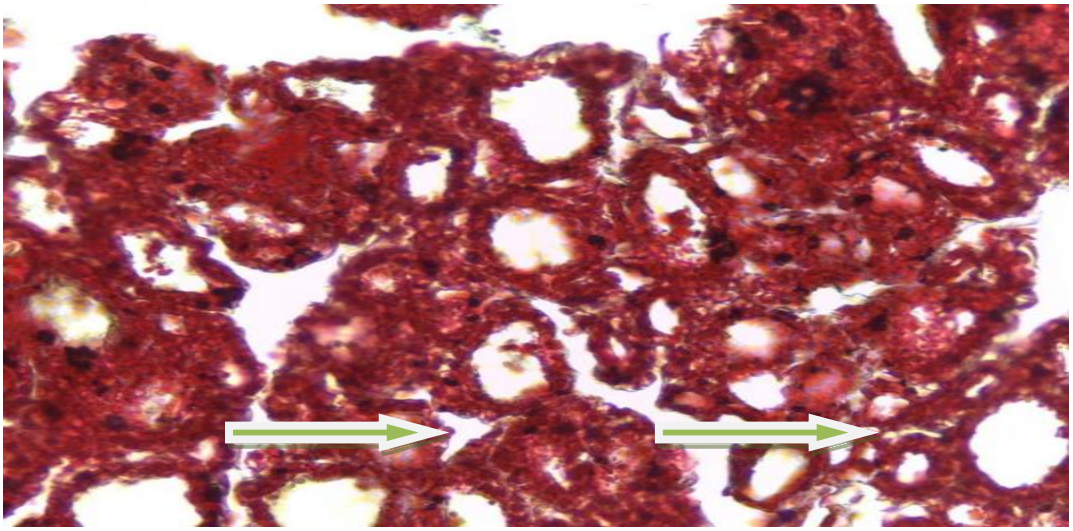


Figure (4-19): Tissue section for thyroid embryo in low dose (0.06 mg/ml) Reveal: small size thyroid follicles, no colloid production, no inflammatory cells infiltration (H & E 400 / X) .

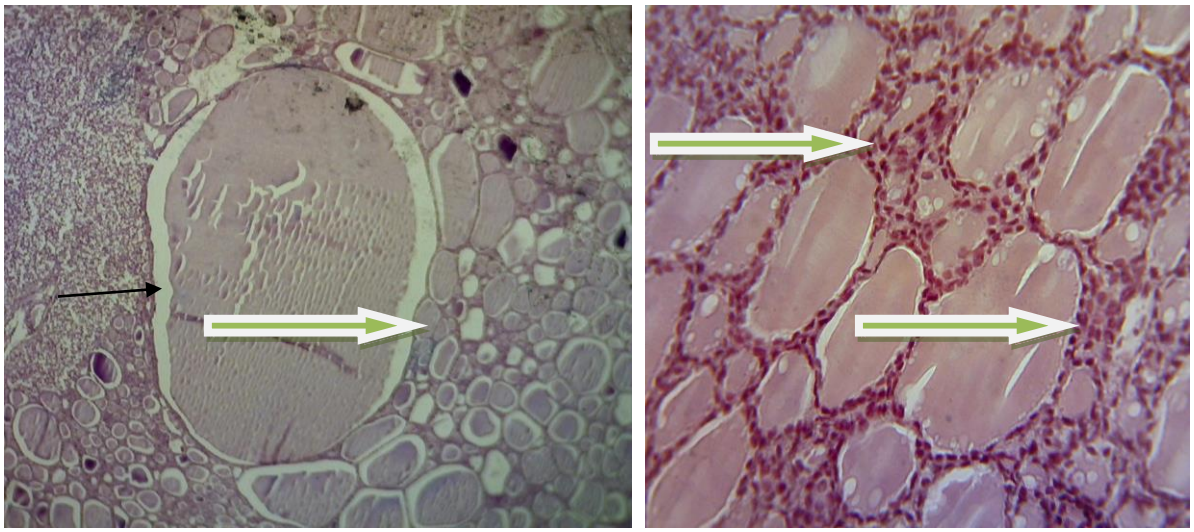


Figure (4-20) : Tissue Section for Thyroid Mother's in low dose (0.06 mg/ml) Reveal: variable size thyroid follicles filled with colloid , no inflammatory cells infiltration (H & E40& 400 / X) .

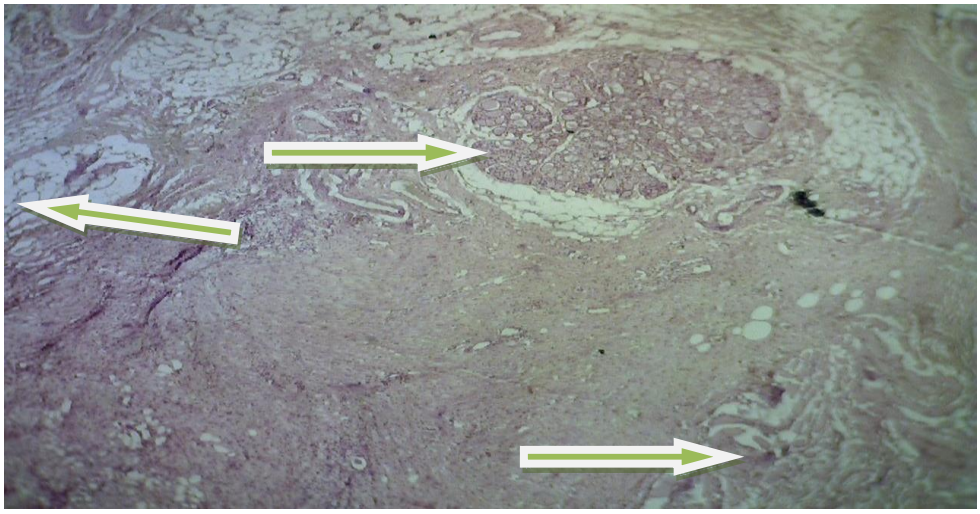


Figure (4-21) : Tissue Section for Thyroid Embryo in Medium dose (1.8 mg/ml) Reveal: small size atrophic thyroid follicles, no colloid production, no inflammatory cells infiltration (H & E 400 / X) .

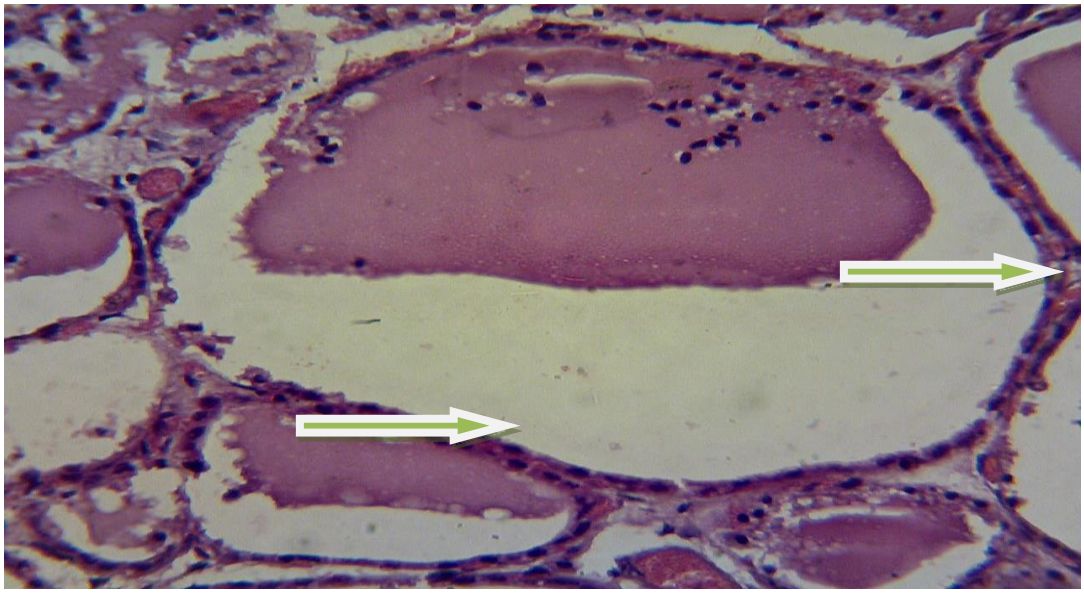


Figure (4-22): Tissue Section for Thyroid Mother's in medium dose (1.8 mg/ml) Reveal: variable large size thyroid follicles filled with colloid ,no inflammatory cells infiltration (H & E 400 / X) .

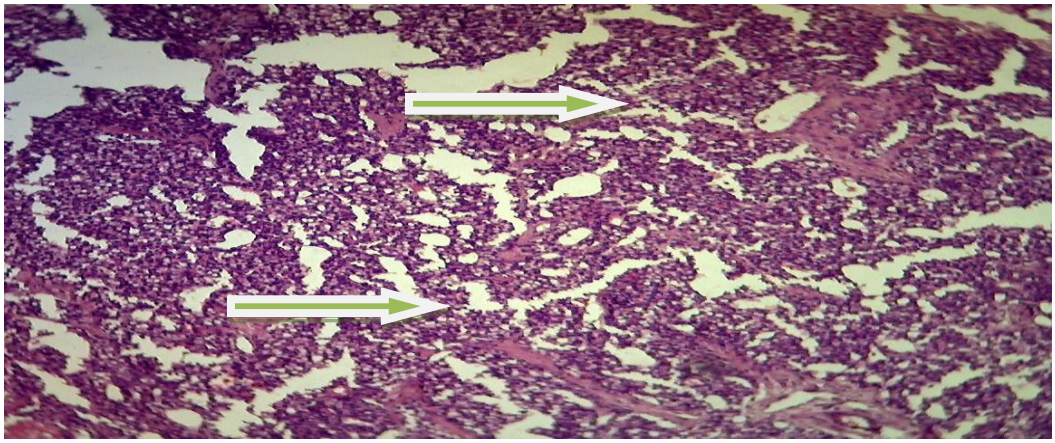


Figure (4-23) : Tissue Section for Thyroid Embryo in high dose (5 mg/ml) Reveal: very small size atrophic thyroid follicles, no colloid production, no inflammatory cells infiltration (H & E 400 / X) .

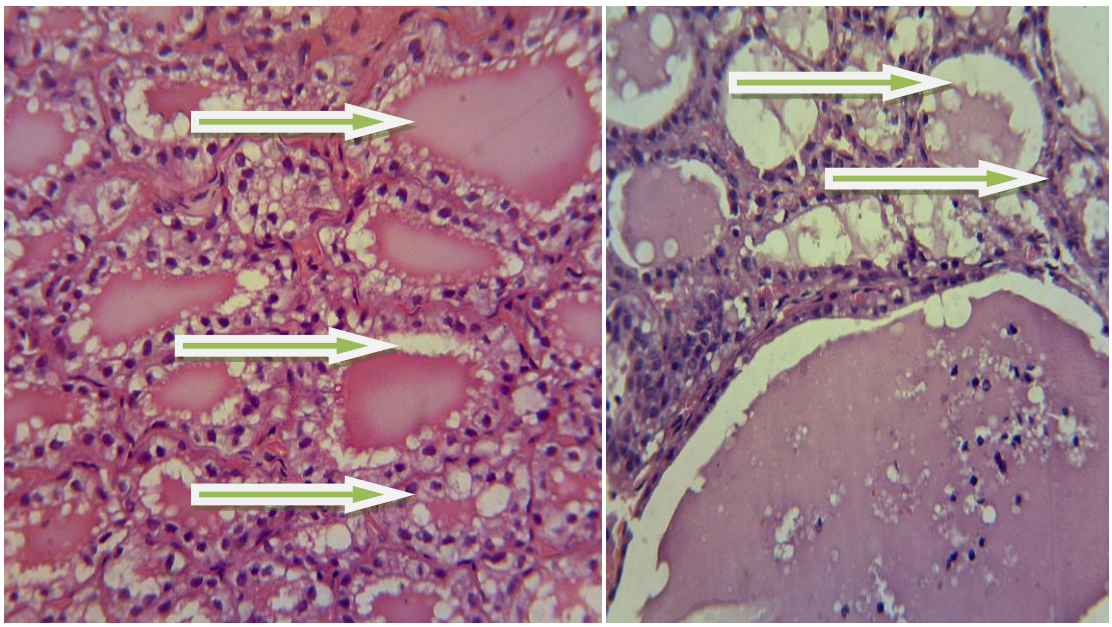


Figure (4-24) : Tissue Section for Thyroid Mother's in high dose (5 mg/ml) Reveal: variable large size follicles lined by active columnar follicular cells with excessive colloid production with scalloping of colloid, no inflammatory cells infiltration (H & E 400 / X) .

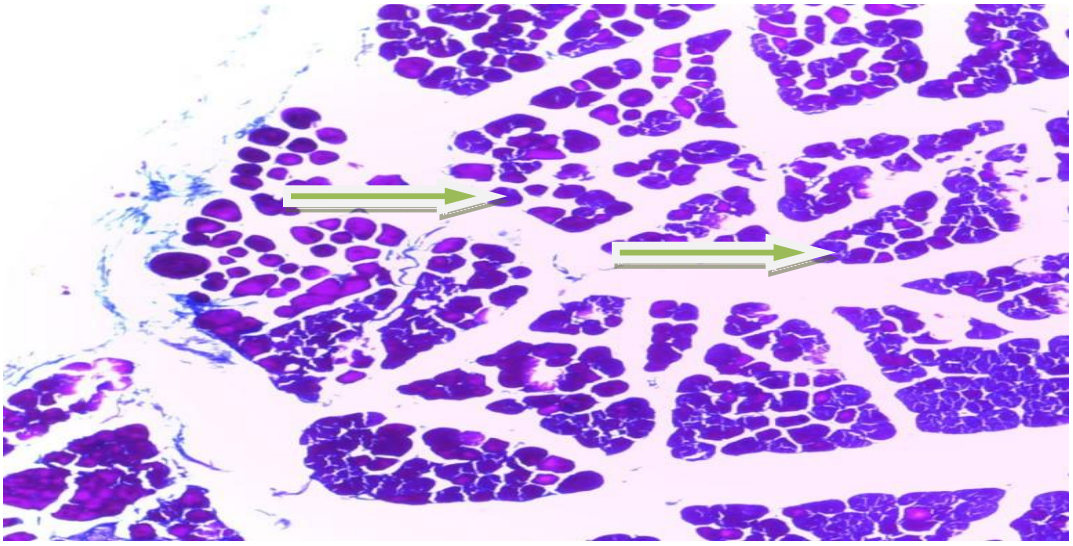


Figure (4-25) : Tissue Section for Thyroid Embryo in very high dose (20 mg/ml) Reveal: very small size atrophic thyroid follicles, no colloid production, no inflammatory cells infiltration (H & E 400 / X).

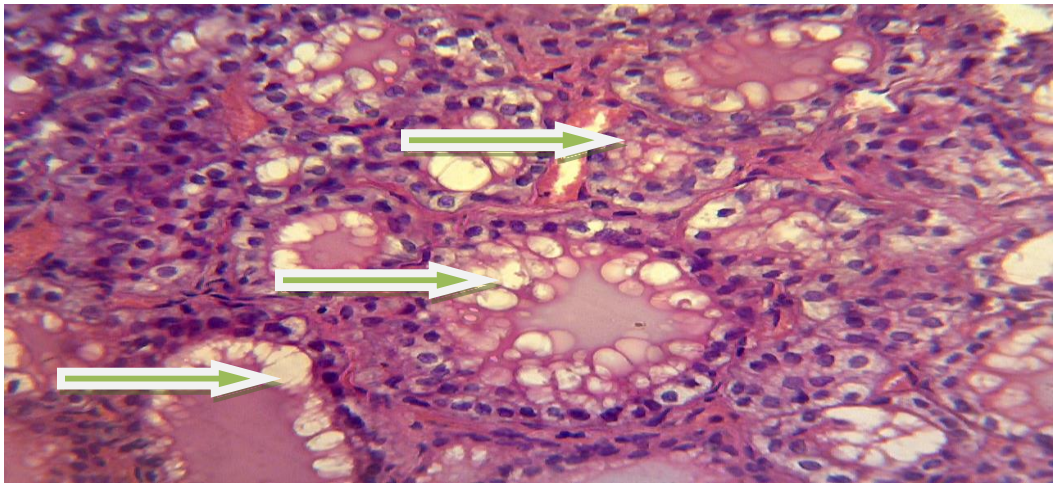


Figure (4-26): Tissue Section for Thyroid Mother's in very high dose (20 mg/ml) Reveal: variable large size follicles some cystic lined by active columnar follicular cells with excessive colloid and scalloping colloid with follicular cell proliferation, no inflammatory cells infiltration (H & E 400 / X).

Our study for the Iodine excess on the thyroid and revealed the macroscopic appearance of thyroid lobes was not significantly changed by Iodine excess exposure during pregnancy and lactation (data not shown), even so the histological analysis has shown some differences among the groups Iodine excess -treated animals presented follicles with various size and Conversely, rat dams exposed to the highest dose of Iodine excess treatment (20×) presented thyroid follicles with slightly increased diameter filled with higher content of TG compared with control group but no infiltration of immune cells was observed in any of the studied groups the results agreed with (Serrano-Nascimento *et al.*,2017) .

Our finding agree who study protective role of selenium on thyroid morphology in iodine-induced autoimmune thyroiditis in rats, thyroid morphology showed that administrated with potassium iodine (KI) group had higher mean value of thyroid follicles and the highest mean value of the thyroid follicular epithelium than control group and in rats, significant differences were found between groups in KI group, severe vascular congestion was observed in 66.7% of mother's rats with resorption vacuoles , and interfollicular spaces, moderate fibrosis according to recent research (Vasiliu *et al.*,2020) .

Iodine exposure leads to increased iodination of thyroglobulin and, therefore, to increased antigenicity (immunogenicity) by creating new iodine-containing epitopes or by discovering cryptic epitopest, this facilitates antigen exposure (antigen processing or antigen presentation) and thus increases T cell receptor binding and activation of T cells, respectively according to research (Rose *et al.*,2002).

Exposure for increased iodine determines the increase of reactive oxygen species (ROS) in thyrocytes, ROS may increase the expression of intracellular adhesion molecule 1 in the thyroid follicular cell, which subsequently attracts immunocompetent cells to the thyroid gland, the binding of ROS to the phospholipidic membrane may induce injury to the thyroid and the release of auto-antigens ; in addition, iodine excess promotes apoptosis of thyroid follicular cells by inducing the expression of TRAIL (TNF-related apoptosis-inducing ligand) necrosis factor and its receptor, death receptor-5, in the thyroid, there is also *in vitro* evidence of the iodine's influence on the immune system cells as it may increase dendritic cell maturation, and increase the number of T cells, as well as the production of immunoglobulins according to this study (Fountoulakis *et al.*,2007).

From other hand explained our study the effect of iodine on the thyroid gland revealed the most of the follicles appeared normal with their simple cuboidal epithelial lining and filled with colloid except for peripheral vacuolation of colloid in some follicles and amount of collagen fibers ;while the effect of excess iodine cause height of the follicular columnar epithelium and the mean area percentages of colloid is significantly increased, the excessive increase iodine may lead to toxic effects were suggested by researchers to be due to its inhibitory action on superoxide dismutase and glutathione peroxidase, the major two enzymes responsible for scavenging harmful reactive oxygen species (ROS) agreement with a study by (Agarwal *et al.*,2006 ; Elswaidy *et al.*,2022) .

Our histology study that investigated induces hyperthyroidism as revealed histopathological alterations in thyroid gland in comparison with the control group in case histochemical examination of thyroid gland of control group show the intensity and homogenous staining of colloid while the thyroid gland of mother's treated groups of excess iodine with (0.06 and 1.8 mg/ml) for 60 days show increased of normal distribution of glycoprotein inside follicles ,the non homogenous cord like materials and some raise intensity of colloid also the thyroid gland of mother's treated with (5 and 20 mg/ml) for 60 days show very high density of colloid follicles epithelium and homogenous colloid materials inside follicles ,congested blood vessels, mention that because inhabit T3, T4 secretion from thyroid gland in case of hyperthyroidism is made to exert a negative feedback of TSH synthesis and decrease secretion , this hormone stimulate the vascularisation of thyroid gland and consequently the gland enlarged , THs increase blood flow to thyroid gland and other organs and tissues so this may cause destruction to the blood vessels results hemorrhage in thyroid tissue, agreement with (Novis *et al.*,2002 ; Mehson *et al.*,2017)

4.8 Histological Examination of Embryo and Mother's Brain.

The microscopic Histological examine In control groups for mother's and embryo brain showed normal histological appearance normal structure tissue (figure: 4-27 & 4-28) ,but the treated groups for mother's and embryo brain with (0.06 mg/ml) showing variable mild of cellular vacuolization and intercellular edema for embryo and very mild reversible paranchymal injury as intercellular edema (prevascular edema) for mother's as show in (figure: 4-29 & 4-30).

Also in groups treated with (1.8 mg/ml) showed the vacuolated neuropils and nerve cell necrosis in brain embryo while variable mild reversible paranchymal injury as cellular swelling , cellular vacuolizaition and intercellular edema for mother brain as show in (figure: 4-31 & 4-32), but in groups treated with (5 & 20 mg/ml) reveal neurons with pyknotic nuclei ,sponge form tissue, congested blood vessels, microglial reaction and eosinophilic neurons in embryo brain while the neurons with pyknotic nuclei with sponge form tissue , eosinophilic neurons, paranchymal injury as cellular swelling , cellular vacuolizaition and intercellular edema in mother's brain as show in (figure : 4-33,4-34,4-35 and 4-36) .

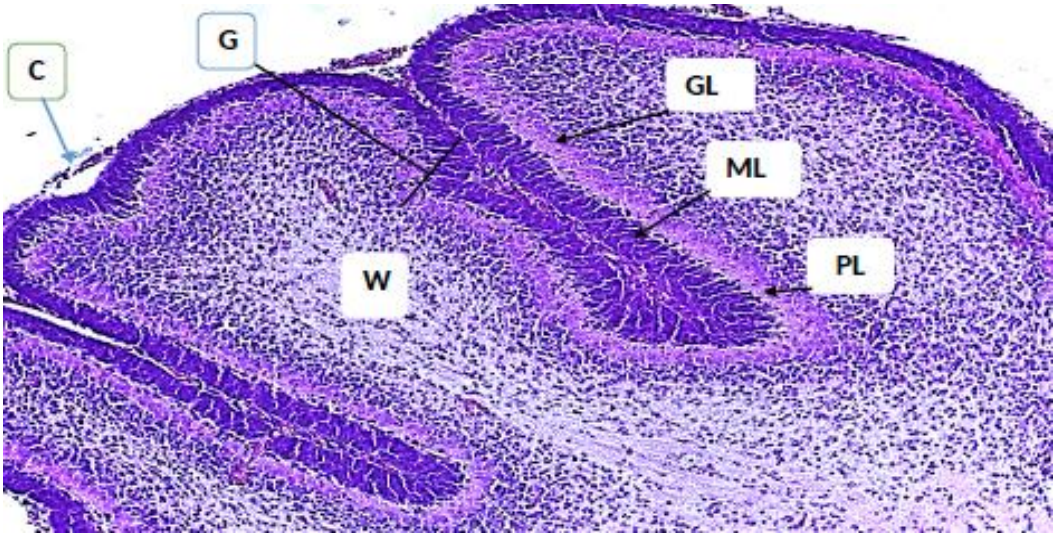


Figure (4-27) : Tissue Section for Brain Embryo Control (Cerebellum part) Show: C: capsule, G: gray matter, W: white mater, GL: granular layer, ML: molecular layer and PL: purkinje layer (H & E 40 / X) .

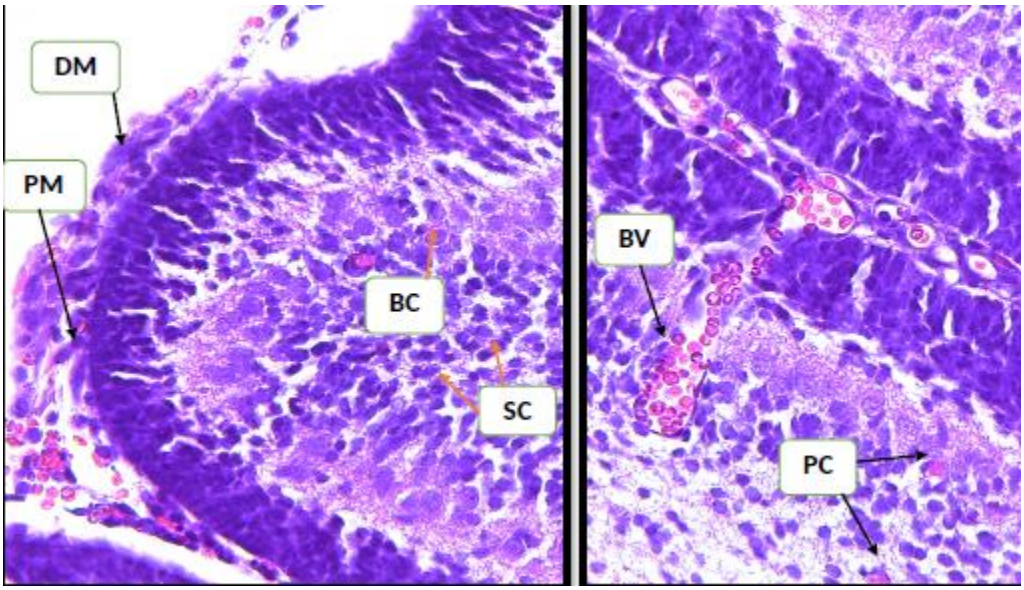


Figure (4-28) : Tissue Section for Brain Mother's Control (Cerebellum part) Show: DM: dura mater, PM: pia mater, BC: Basket cells, SC: Stellate cells, PC: Purkinje cells and BV: Blood vessels with RBC (H & E 200/ X) .

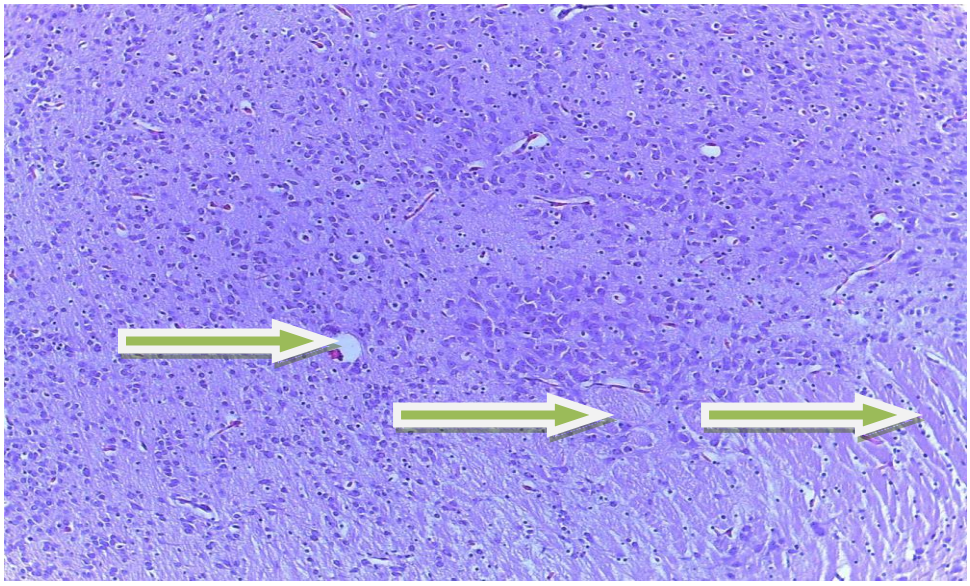


Figure (4-29) : Tissue Section for Brain Embryo in low dose (0.06 mg/ml) Show: no clear pathological changes in the variable mild of cellular vacuolization and intercellular edema (H & E 400 / X) .

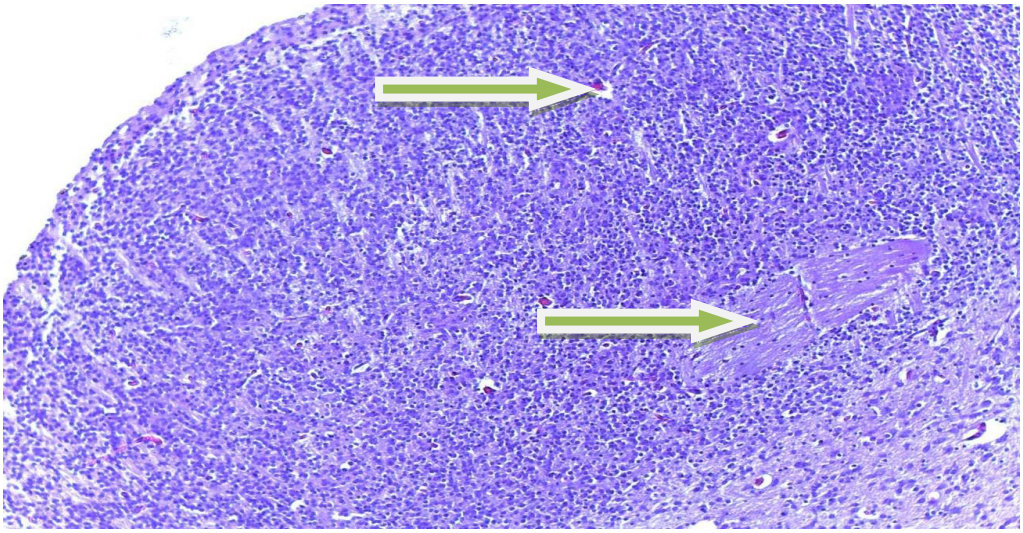


Figure (4-30) : Tissue Section for Brain Mother's in low dose (0.06 mg/ml) Show: very mild reversible paranchymal injury as intercellular edema (prevascular edema) (H & E 100 / X).

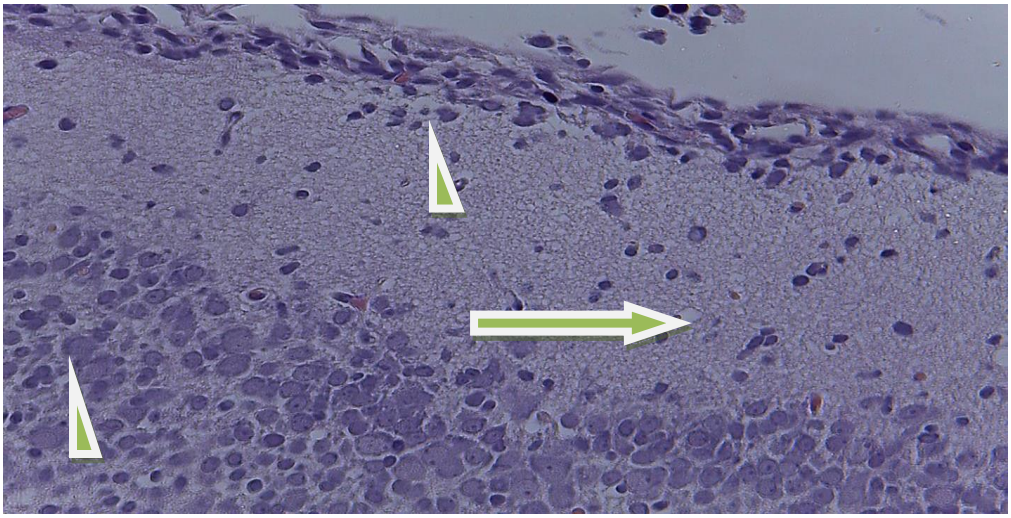


Figure (4-31): Tissue Section for Brain Embryo in medium dose (1.8 mg/ml) Show: the vacuolated neuropils (arrow) and nerve cell necrosis (arrowhead) (H & E 200 / X) .

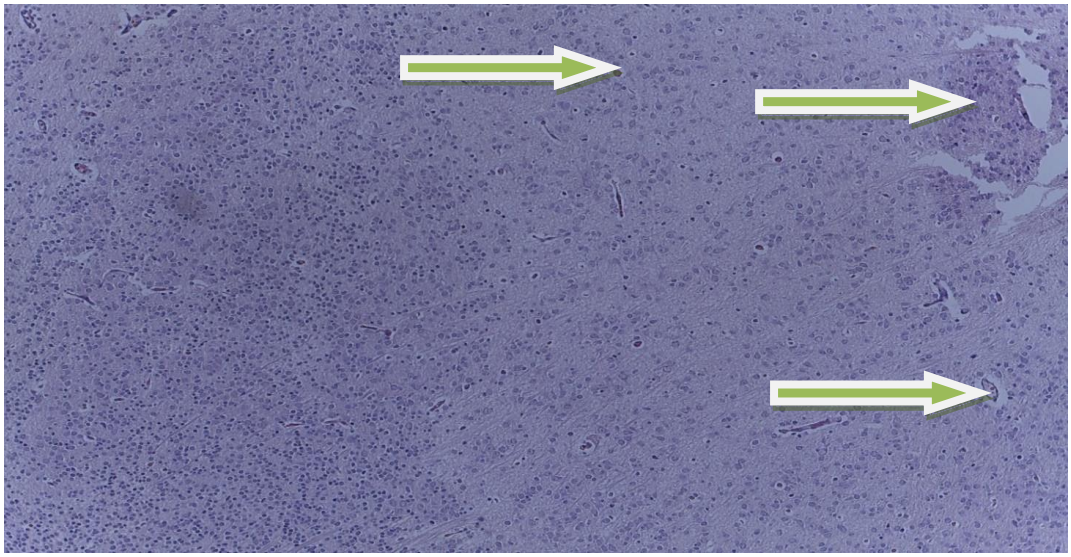


Figure (4-32): Tissue Section for Mother's Brain in medium dose (1.8 mg/ml) Show: variable mild reversible paranchymal injury as cellular swelling , cellular vacuolizaition and intercellular edema (H & E 200 / X) .

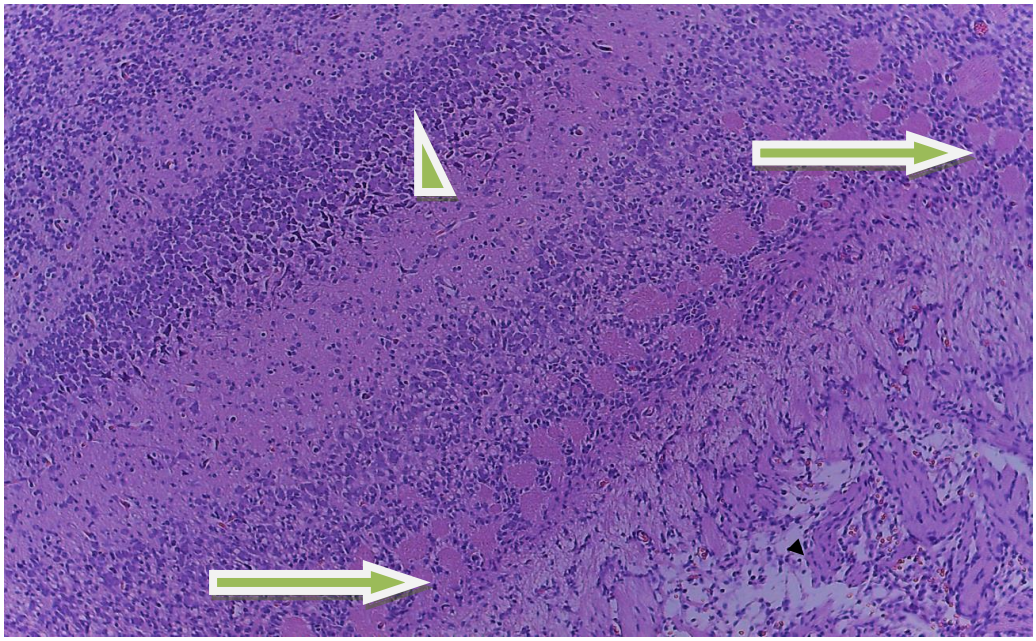


Figure (4-33): Tissue Section for Brain Embryo in high dose (5 mg/ml) Show: the neurons with pyknotic nuclei (arrow) with sponge form tissue and eosinophilic neurons (arrowhead) (H & E 400 / X) .

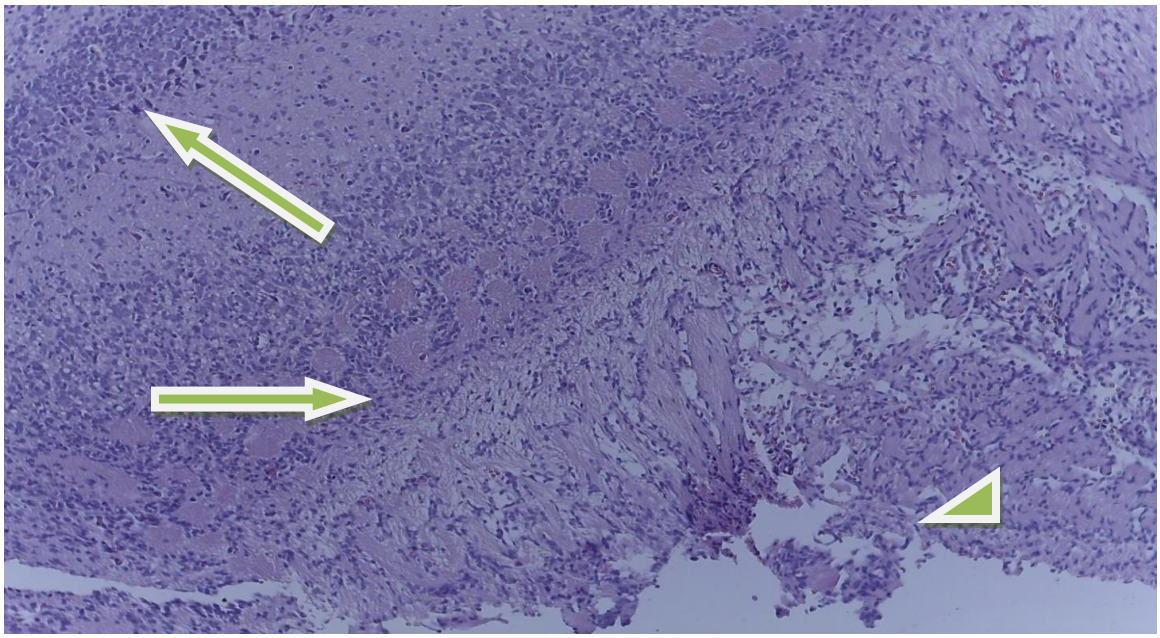


Figure (4-34): Tissue Section for Mother's Brain in high dose (5 mg/ml) Show: the neurons with pyknotic nuclei (arrow) with sponge form tissue and eosinophilic neurons (arrowhead) (H & E 100 / X) .

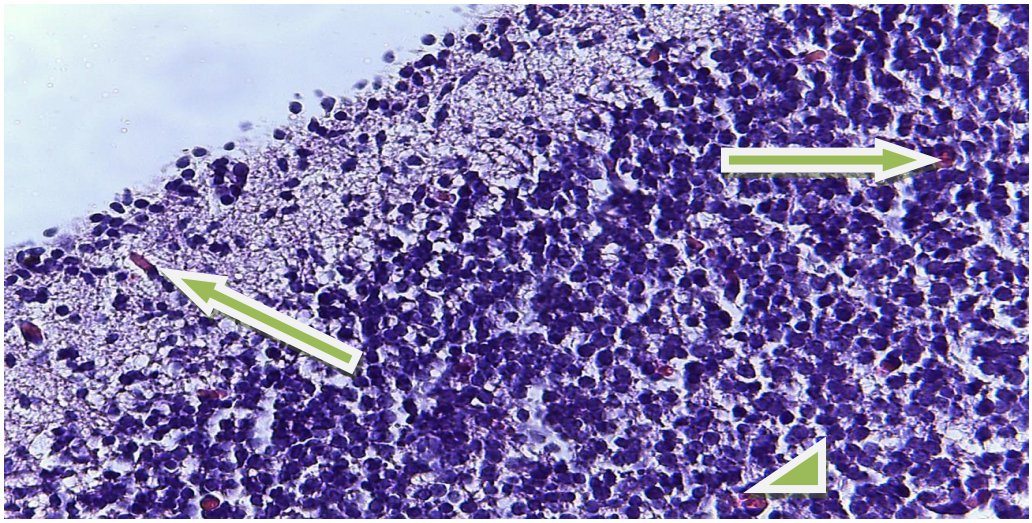


Figure (4-35): Tissue Section for in Embryo Brain very high dose (20 mg/ml) Show: congested blood vessels (arrow) and severe microglial reaction (arrowhead) (H &E 400/ X).

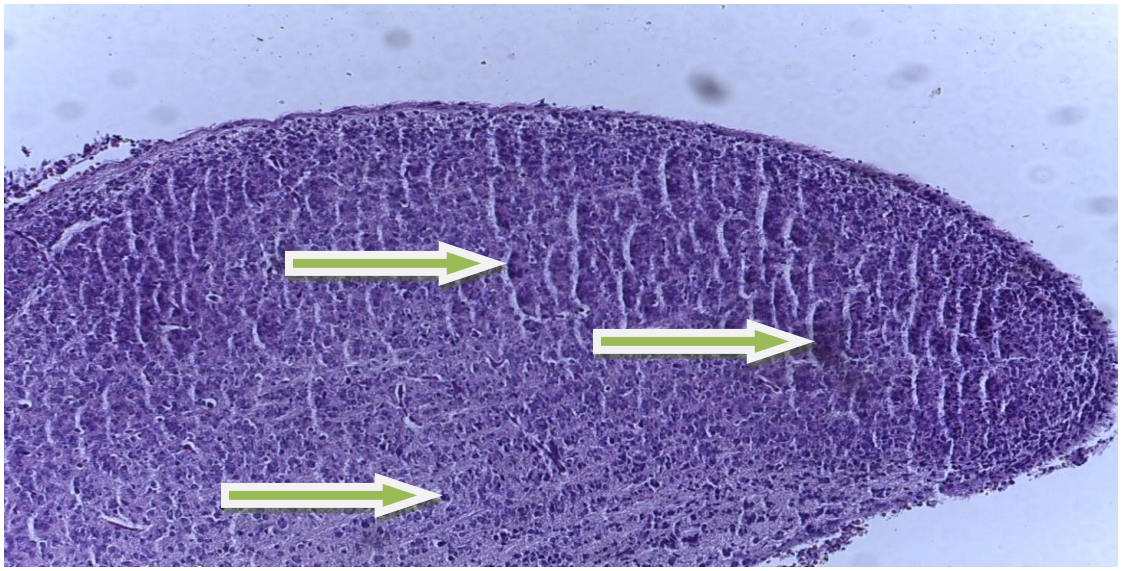


Figure (4-36): Tissue Section for in Mother Brain very high dose (20 mg/ml) Show: variable mild changes of paranchymal injury as cellular swelling , cellular vacuolizaition and intercellular edema (H &E 40/ X).

Our study found in histological exam for control groups vesicular nuclei of the pyramidal cells were visible, blood capillaries were found in the molecular and polymorphic layers but for treatment group with excess iodine showed different changes in brain tissue of mother and embryo according amount the dose of iodine and when increase dose raise the damage in the formation of tissue special for fetus and discovered vacuolation in the external granular layer, delayed its disappearance, and Purkinje cell degeneration that grew worse with age that explain the children's levels of myelination, neurofilament concentration, and Reelin gene expression were lower than normal, the developmental hypothyroidism for children's is known to result in long-lasting abnormalities in cerebellar development and our study found that maternal hyperthyroidism during pregnancy and breastfeeding caused developmental problems in the cerebellar cortex of their offspring, as shown by the results. Large parts of the underlying molecular pathways were unknown; Reelin is a crucial molecule that affects cerebellar development and neuronal migration the study also concurred with (Mohamed and Ahmed, 2018; Elkholy *et al.*, 2021).

The number of neurons, dendritic arborization, and synaptogenesis are reduced and neuronal migration to the hippocampus and neocortex regions in rodents is altered by experimentally induced thyroid hormones deprivation during sensitive phases of the fetus's brain development and by affecting neuronal migration, thyroid hormones disorder during early pregnancy causes effect clearly defined cortical layering in mice also affects the cytoarchitecture of the hippocampus and the somatosensory cortex according to recent research (Gilbert *et al.*, 2012).

Our study proves the pregnancy-related changes in DNA hypermethylation of several important brain genes, including BDNF, such as induced hypothyroidism in mice affect a variety of brain functions, including hippocampal-dependent learning and memory and neural plasticity processes, in

humans, even a mild subclinical maternal hypothyroxinemia, especially in the early stages of pregnancy, results in delayed motor and mental development as well as lasting brain damage our study support and agreement with (Pop et al., 2004; Gilbert et al., 2012).

Out of all the cognitive components, memory is the one that is most severely compromised, as evidenced by imaging studies of the brain that showed reduced hippocampal volume and cerebral blood flow that showed in our study in regions that are involved in attention, visuospatial processing, working memory, and motor speed (Correia *et al.,* 2009). A few hypo-hyperthyroid adult patients have also reported neuropsychiatric manifestations like anxiety, depression, and reduced cognition (Samuels *et al.,* 2014).

Our study proves the thyroid hormones crucial for brain development, although the precise underlying process is yet unknown, emerging data suggests that one of the contributing factors may be low levels of BDNF expression, as this neurotrophin is essential for the development of synapses, memory, etc according to a variety of studies particularly (Kasatkina *et al.,* 2006; Chakraborty *et al.,* 2012).

Conclusion

1- Depending on the obtained results, we were found the excess iodine before and during pregnancy has a direct effect on the thyroid hormones for the mother that lead to raise the percent of thyroid hormones (T3, T4 and TSH).

2- According of the current staty the thyroid transcription factors and brain parameters were involved directly with excess iodine taken, which all the factors were evaluated with iodine doses in both pregnant mother and embryos rats.

3- Depending on the obtained results, we were found the excess iodine before and during pregnancy has a direct effect on the reduce the weight and reproduction for the mother and embryo.

4- According our data the iodine increasing before and during pregnancy may be one of causes of histological changes in mother and embryo thyroid and brain tissues .

List of contents

List of Abbreviations

ID	Iodine deficiency
IE	Iodide effect
TH	Thyroid hormones
IQ	Intelligence quotient
DIT	Di-iodotyrosine
KI	Potassium iodide
ELISA	Enzyme-linked immune sorbent assay
D1	Deiodinase
MDA	Malondialdehyde
TPO	Thyroid peroxidase
TT3	Total triiodothyronine
TT4	Total thyroxine
FT4	Free thyroxine
FT3	Free triiodothyronine
OS	Obese strain
NADPH	Nicotinamide adenine dinucleotide phosphate subunit protein
ROS	Reactive oxygen species
NIS	Sodium-iodide(Na+ / I) Symporter
TxnRd1	Thioredoxin reductase-1
Tg	Thyroglobulin
SSRIs	Selective serotonin reuptake inhibitors
AChE	Acetylcholinesterase
CH	Congenital hypothyroidism
TSH	Thyroid-stimulating hormone
RMR	Resting metabolic rate
TTFs	Thyroid transcription factors
WHO	World Health Organization
BDNF	Determination of Rat Brain derived neurotrophic facor
ACH	Determination of Rat Acetylcholine
PAX-8	Paired box protein
FOXE1	Forkhead box protein E1
Nkx-2.1	Homeobox protein
NICE	National Institute for Health and Care Excellence's
rT3	Reverse Triiodothyronine hormone
BMIC	Breast milk iodine content
SMVT	Sodium multivitamin transporter
CFTR	Cystic fibrosis transmembrane conductance regulator

T3	Triiodothyronine hormone
T4	Thyroxin hormone
UIC	Unit Identification cod
TBG	Thyroxin-Binding Globulin
RNI	Recommended nutritional intake
CT	Cortical thickness
SA	Surface area
CSF	Cerebrospinal fluid
AIT	Autoimmune thyroiditis
TRH	Thyrotropin-Releasing Hormone
GR	General Reaction
TSH	Thyroid Stimulating Hormone (thyrotropin)
DEHAL1	Iodotyrosine Dehalogenase 1
MCT8	Monocarboxylate Transporter8
TSHR	Thyroid Stimulating Hormone Receptor
RGC	Retinal ganglion cell
IPC	Infection Prevention Control

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Recommendations

1- Conducting a study to find out the effect of iodine on the reproductive system from the histological and physiological aspects of males and females.
2- Conducting a study of the physiological changes of embryo after birth and measuring the percentage of the effect on hormones.
3- Studying the effect of iodine on the functions of other parts of the body, such as the pancreas, liver and kidneys.
4- Accounted in this study before and after birth to know the effect of the increase in iodine transmitted through milk compound .
5- Conducting a study to find out the relationship between occur mutations and effect on genes with iodine and thyroid function .

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