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Histopathological effects of retinol on blastocyst implantation in pregnant rats

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***Abstract*---**Retinol is lipid soluble vitamin widely used during pregnancy as a food supplement.The study has been conducted in Animal House/Faculty of Sciences/University of Kufa between December 2021 and February 2022, fifteen female Albino Rats are used. The present study has been intended to show the histolopathologiacl effects of retinol on blastocyst implantation in female Albino Rats. The females Rats are randomly divided into three main groups, comprising five rats for each group. The control group is given orally injection of physiological normal saline and the second and third group are given orally injection of retinol doses ten, twenty µcg/kg/day respectively for seven days from the first day to the end of experimental allocated for each female.The rats are sacrificed in 7 th (dpc) to study the implantation of embryos in decidual tissues. Retinol doses ten, twenty µcg/kg/day caused macroscopic malformations in embryos of female rats compare to control group. The histopathlogical study of female rats embryos treated with ten, twenty µcg/kg/day of retinol have shown deformations of decidual tissues zonation.In conclusion; retinol caused malformations of embryos in 7 th (dpc) and histopathlogical effects in decidual tissue zonations of blastocyst.

***Keywords*---**Rats, Retinol, Implantation, Embryos.

Introduction

Retinol is the generic term for a variety of fat-soluble substances including retinol, retinyl palmitate and the provitamin A carotenoids such as all-trans-β-caroteneits, its commonly known as the anti-infective vitamin and has an essential role in vision and cellular differentiation, the latter providing a unique core mechanism helping to explain the influence of vitamin A on epithelial

barriers (Matteoni *et al.*,1999).Retinol adequacy is discussed in terms of the recommended allowances appropriate for the needs of the majority of individuals, deficiency can result in xerophthalmia and permanent blindness and in increased mortality rates among children(Lawrence, 2022).Toxicity has been associated with the overconsumption of retinol supplements, acute hypervitaminosis A may occur after ingestion of greater than or equal to 500,000 IU by adults or proportionately less by children (Mawson and Gonzalez-Fernandez, 2021). Benefits to public health can be expected by improving the retinol status of deficient populations through an appropriate mix of acceptable, affordable, and available programs including promotion of breast-feeding, control of infections, dietary diversification, food fortification, and supplementation. Benefits include not only improved health and welfare for individuals and their families (de Montemor Marçal *et al.*, 2021). Retinol (all-trans-retinol), its active derivatives retinal and retinoic acid, and their synthetic analogues constitute the group of retinoids. It is obtained from diet either as preformed vitamin A or as carotenoids, retinal plays a biological role in vision, but most of the effects of vitamin A are exerted by retinoic acid, which binds to nuclear receptors and regulates gene transcription (Cabezuelo *et al.*, 2020; Hadi and Aljanaby, 2022).Retinol toxicity can due to either topical or oral vitamin A administration. Oral vitamin A toxicity can be acute, due to the ingestion of a large amount of vitamin A over a short period of time, or chronic, due to oral ingestion over a longer duration, the most severe adverse effect of systemic retinoids is teratogenicity (Olson and Goyal, 2021).The most common adverse effect of topical retinol is skin irritation, erythema, and peeling. This activity describes the evaluation and management of retinol toxicity and highlights the role of the interprofessional team in improving care for affected patients(Hu *et al.*, 2021). Animals cannot synthesize vitamin A de novo, they must obtain it either as preformed vitamin A from animal products or as carotenoid precursors from plant sources. Due to its essential role in the visual system, acute vitamin A deprivation impairs photoreceptor function and causes night blindness (poor vision under dim light conditions), while chronic deprivation results in retinal dystrophies and photoreceptor cell death (Dewett *et al.*, 2021; Medhat and Aljanaby, 2022). Tsatsakis *et al.* (2021) study the effects of vitamin A on physiological parameters in rats, after 6 months, ten rats from each group were sacrificed and a complete evaluation of blood and urine biochemistry, biomarkersof oxidative stress , xenobiotic detoxification enzymes and lysosomal enzymes and organ histopathology was performed.

Material and Methods

Animal Model

This study achieved on pregnant white rat *Rattus norvegicus* females (15) and males(5) for mating. All rat weights ranging from 200-250 g. They should be in good health. The rats are placed in plastic cages with metal covers, 48 cm wide, 15 cm wide and 7 cm wide. The sawdust, which should be replaced three times a week, is considered in its care to clean the hatching of the special diet and plastic bottles can be used to make a watering tough with a cork equipped with metal pipes. The animals are placed under suitable laboratory conditions in terms of temperature 18-26 C° and light/dark cycle 10/14 and ventilation rate time/hour 10-15 and also the relative humidity 30-70 (Tan & Tan, 2017).

Vaginal smear

Vaginal smear was collected to identify the different phases of the estrus cycle. At the end of the proestrus, the adult virgin female rats were subjected to males to be fertilized. The presence of spermatozoa in the vaginal smear indicated successful mating and was considered as day one of gestation (Keshri *et al.*, 2003).

The Mating

The process of mating is will do by placing three mature females with one fertile male in each cage throughout the night. It is ascertained that the marriage will be achieved the next morning by observing the existence of the vaginal plug (Nau, 1992), which consists of a mixture of secretions of the vesicular glands and the coagulating glands of the male. The vaginal plug appeared during 16-24 hours after mating and stay for about 48 hours. The proportion of reliance on this method to ensure that the pregnancy occurs range between 80-90%, we have also adopted on detection of pregnants by vaginal smears for detect presence of male sperms in vaginal female where it is a sign of pregnancy(Eveline *et al.,* 2002), depend then isolate females who possess the vaginal plug and vaginal smears containing sperms and the day that was seen vaginal plug and vaginal smears is today zero of pregnancy and the day after which is the first day of pregnancy, the pregnants were then taught and isolated for experimentation.

Drug used

In this study, Retinol is used in the form of capsule 3000 microgram, from AL-Hady Drug Store, MADAMAR Poland Company, which is used orally in human, injection of experiment animals by oral and Intraperitoneal (IP) using disposable syringe. The dose given to the animal is prepared as follows: Retinol concentration 3000 microgram (10000 IU), Suggest human body weight is 70 k = 70000 g. Ingested of 1 ml orally (20 microgram/kg/day) (double dose), Ingested of 0.5 ml orally(10microgram/kg/day) (single dose)(Larson *et al.*, 2003).

Experimental croups

First: Control group: included five female rats injected by normal saline (Nacl 0.9%) orally for seven days, the group sacrifice it in the end of experiment, for knowledge of retinol effect on implantation. Second: first treated group: included five female rats injected by dose 10 microgram/kg/day of retinol orally for seven days, the group sacrifice it in the end of experiment, for knowledge of retinol effect on implantation. Third: second treated group: included five female rats injected by dose 20 microgram /kg/day of retinol orally for seven days, the group sacrifice it in the end of experiment, for knowledge of retinol effect on implantation.

Animals sacrifice and collection of embryos

The experimental animals of all groups were sacrifice after general

Anesthesia by combination of Ketamine: Xylazine (90mg/ kg: 10mg/ kg intraperitoneal), used ketamine 0.5 ml & xylazine 0.1 ml to each 250 g of body weight for anesthesia when sacrifice the pregnant females from the control & treated croups, after the anesthesia the pregnant females of rats put in anatomical dish and made linear incision by scissors in abdominal region for extraction the uterus horns that contains the embryos for collected, and ovaries, lives, kidneys, spleen , then removed the organs & embryological membranes by anatomical tools. Saved in containers contains 10% formalin (AlTameemi, 2014).

Histological preparations

Done samples saved after remove them from animals in containers contains 10% formalin (38%100ml formalin in 900ml tap water) and then done series of operations depending on the method described in (Suvarna *et al.*,2013).

Staining and mounting
Used the following special stains to colorize slides of different types of tissue:

Harris Hematoxylin stain
A general base color used to color the nucleus in dark blue color.

Eosin stain
A general acidic color used to color the cytoplasm in dark red color.
(Suvarna *et al* .,2013).

Results and Discussion

Macroscopic Observations

Macroscopic Features of Uterus, Ovaries, and Embryos 7th Day Post Coitum (dpc). The uterus of pregnant rats (7th dpc) in control showed more regular distribution of embryos and natural bloody form, no stillbirth, while treated groups showed little numbers of embryos with pale form, stillbirth, the uterus horns have (V) shaped connect with ovaries, the uterus from rats treated with retinol appeared in different distribution of embryos than normal in which the horns loaded with pale and small embryos (Figures 1 and 2). The ovaries in pregnant rats of control and treated groups with retinol look normal and have oval shape, regular and granular surface surrounded with connective adipose tissue and attached to uteri horns (Figures 1 and 2). The defects caused may be by vitamin A toxicity in uterus tissue which effect on functional role of blood processing of blastocyst.All-*trans* retinoic acid (RA), the oxidative metabolite of vitamin A, is essential for normal development. In addition, high levels of RA are teratogenic in many species. We have previously shown that excess RA results in immediate effects on the preimplantation embryo and on blastocyst development. This study was conducted to clarify the long-term survival of mouse blastocyst and the effect of RA on gene expression.we identified the immediate adverse impact of RA on mouse blastocyst development. This involved an inhibition of cell proliferation and growth retardation. Using an *in vivo* model, we also identified the resorption of postimplanted blastocysts that had been treated with excess RA.This is the first evidence to show the impacts of RA on mouse blastocysts *in vitro* and

any carry-over effects in the uterus. There is a retardation of early postimplantation blastocyst development and then subsequent blastocyst death. Our findings also show that there is some degree of selective induction of retinoic acid receptors when excess RA is administered to the blastocysts (Huang *et al.*,2006). The results showed that the effects of the teratogen on the cultured embryos were similar to those on the embryos allowed to continue development for the same period in the mother. In both groups RA reduced protein synthesis, inhibited somite and limb bud formation, and caused various neural tube defects, particularly microcephaly and abnormalities in the closure of the anterior and posterior neuropores (Steele *et al.*,1983).vitamin A suggests toxicity and synergism with vitamin A. These results suggest that caution must be taken when taking these extracts during pregnancy due to their possible toxicity and teratogenicity (Herrera *et al.*,2011).Strong immunostaining for RBP and hybridization signals for RBP mRNA were observed in trophectoderm of tubular but not spherical blastocysts. RBP mRNA was localized in epithelial cells lining the chorion, allantois, and amnion. In addition, RBP mRNA was detected in cotyledons, the sites of chorionic attachment to the uterine endometrium and physiological exchange between the embryo and its mother. Expression of RBP in expanding conceptuses, developing extraembryonic membranes, and sites of fetal-maternal attachment suggests that the extraembryonic membranes regulate retinol transport and availability within the conceptus (Liu *et al.*,1993).

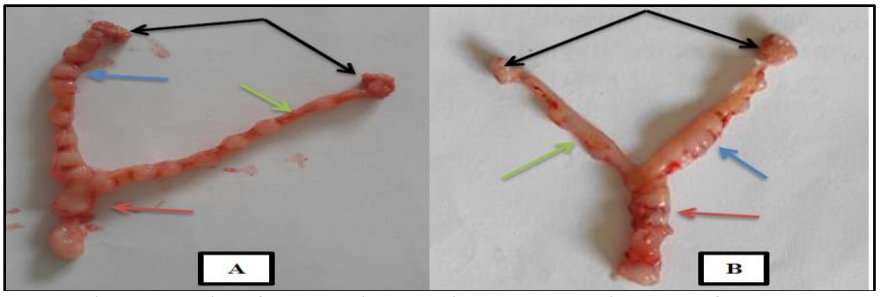

Figure 1: A- Photograph of normal complete uterus horns of pregnant rat (7th dpc) control group.B- treated dose 10µ/kg/day of retinol show vagina ➔, uterine horns ➔, embryos ➔ and ovaries ➔ .

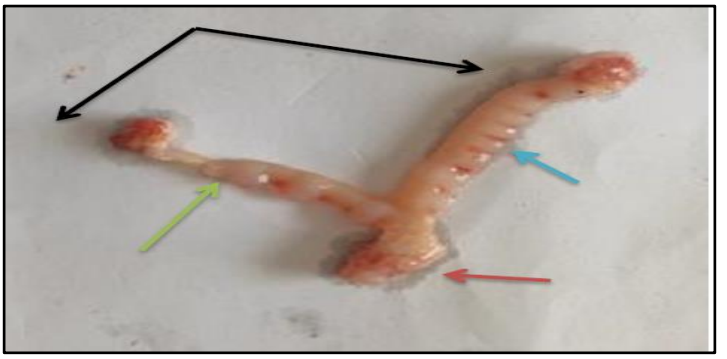

Figure 2: Photograph of complete uterus horns of pregnant rat (7th dpc) treated dose 20 µ/kg/day of retinol show vagina , uterine horns , embryos and ovaries .

Histological Sections Staining by Hematoxylin and Eosin
7th Day Post Coitum (dpc)

The days post coitum is a term commonly used in medicine and biology to refer to the age of an embryo.The results have showed colored sections by H&E taken from the implantation sites of treated group by retinol for 7th day post coitum results different to control group. The first event is stromal cells in endometrium that suffer modifications to converted to decidual tissue, which is an important indicator of successful implantation in antimesometrial side. In treated groups all zonation (PDZ, SDZ, UBZ, IZ, UBZ) which affected. In sections staining by H&E it is possible to identify four main zones (Figures 3, 4 and 5).

1- Primary decidual zone (PDZ): The cells of decidual tissue are thick and compact which surrounds the blastocyst and endometrium (Figure 3). not observed any blood vessels in this zone.
2- Secondary decidual zone (SDZ):The zone which is located between the (PDZ) and Undifferentiated basal zone (UBZ),possible observed blood vessels and intercellular spaces.
3- The implantation Zone (IZ): Its small zone which surrounds and adjacent the emberyo in the side of endometrium in Antimesometrial site.
4- Undifferentiated basal zone (UBZ): Its located between the (SDZ) and myometrium, small cells compared to (PDZ) and (SDZ).

So In vertebrates and invertebrate chordates, RA has a pivotal role during development, altering levels of endogenous RA signaling during early embryology, both too low and too high, leads to birth defects, including congenital vascular and cardiovascular defects. Of note, Fetal Alcohol Spectrum Disorder encompasses congenital anomalies, including craniofacial, auditory, and ocular defects, neurobehavioral anomalies and mental disabilities caused by maternal consumption of alcohol during pregnancy (Marlétaz *et al.*,2006).Altering levels of endogenous RA signaling during early embryology leads to severe malformations, mainly due to incorrect positional codes specifying the embryonic anteroposterior body axis. In this review, we present our current understanding of the RA signaling pathway and its roles during chordate development (Littlewood *et al.*,2006). The mechanism of RA effects on decidaul tissues to transport to stromal cells is not understood. Ozaki *et al.*,(2017) discovered RA effect on signaling and metabolic pathway.

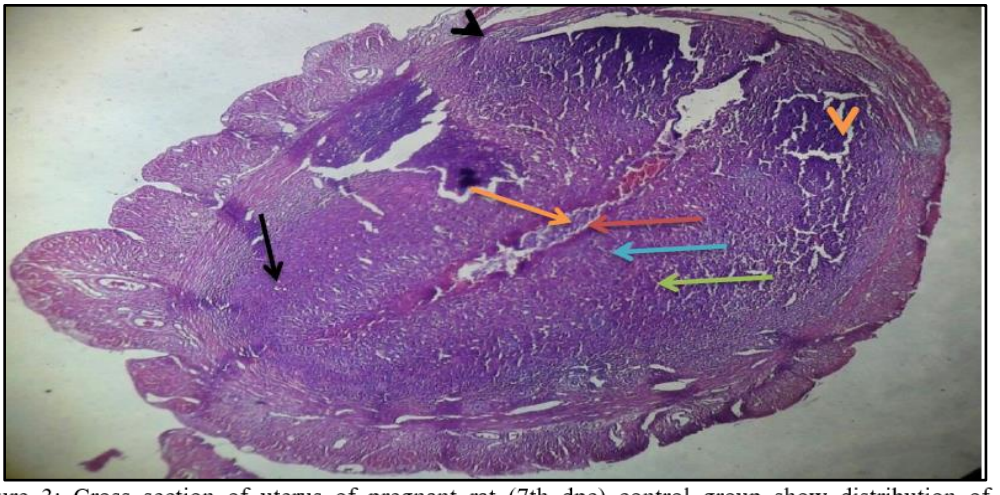

Figure 3: Cross section of uterus of pregnant rat (7th dpc) control group show distribution of decidual tissue zones in endometrium and interaction embryo , primary decidual zone , secondary decidual zone the implantation zone , mesometrial site , antimesometrial site , undifferentiated basal zone . H&E, 4X.

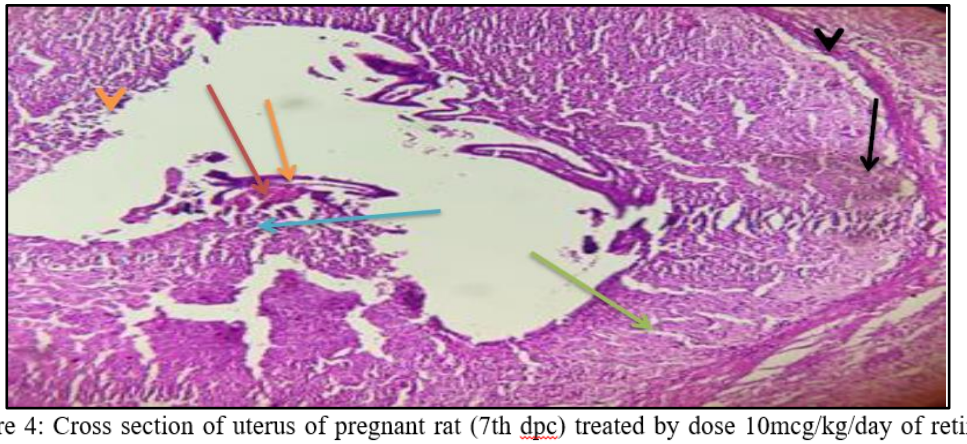

Figure 4: Cross section of uterus of pregnant rat (7th dpc) treated by dose 10mcg/kg/day of retinol show distribution of decidual tissue zones in endometrium and interaction embryo , primary deciual zone , secondary decidual zone , the implantation zone , mesometrial site , antimesometrial site , undifferentiated basal zone , H&E, 4X.

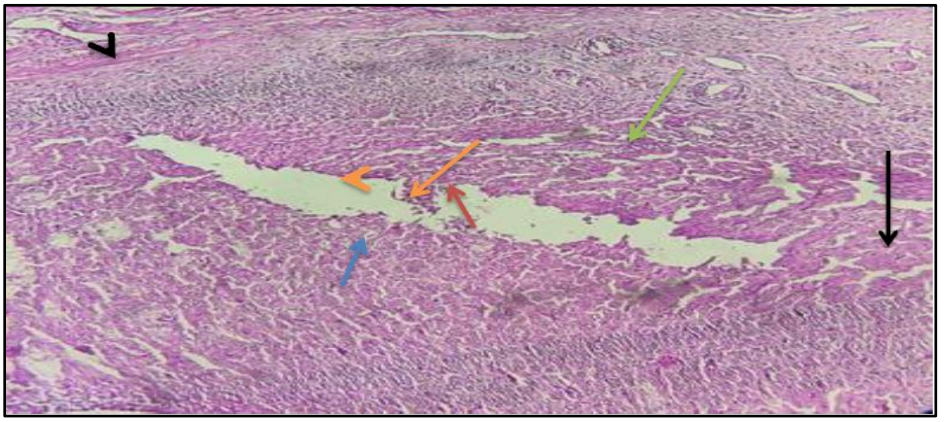

Figure 5: Cross section of uterus of pregnant rat (7th dpc) treated by dose 20mcg/kg/day of retinol show distribution of decidual tissue zones in endometrium and interaction embryo →, primary decidual zon →, secondary decidual zone →, the implantation zone →, mesometrial site →, antimesometrial site →, undifferentiated basal zone → blood sinusoids to mesometrial site →.H&E, 4X.

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