EFFECT OF GRAPES (*Vitis vinifera*) JUICE ON THE THREE STAGES OF PREGANCY IN ALBINO WISTAR RATS

BY

Favour Gesere MAXWELL (Miss)

LSC2007314

DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

OCTOBER, 2025

EFFECT OF GRAPES (*Vitis vinifera*) JUICE ON THE THREE STAGES OF PREGANCY IN ALBINO WISTAR RATS

BY
Favour Gesere MAXWELL (Miss)

LSC2007314

DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

OCTOBER, 2025

CERTIFICATION

This is to certify that this project titled "Effect of *Vitis Vinifera* on three stages of pregnancy development ''
was carried out by **Favour Gesere MAXWELL,** with matriculation number LSC2007314, of the Department of Science Laboratory Technology (Physiology/Pharmacology), Faculty of Life Sciences, University, Benin City, Edo state, Under the supervision of DR. P. O. OBARO.

DR .P.O. OBARO
PROJECT SUPERVISOR

Date

DR. ALONGE
PROJECT COORDINATOR

Date

PROF. J. O. OSARUWMENSE
HEAD OF DEPARTMENT

Date

EXTERNAL EXAMINER

Date

DEDICATION

This work is dedicated to God almighty, my family, my supervisor and everyone who supported me throughout the course of this work.

ACKNOWLEDGEMENT

My profound gratitude to God almighty for his faithfulness. To my supervisor DR. P.O. OBARO and his wife for their guidance and assistance.

I am grateful to my parents Mr. and Mrs. Maxwell Irowainu and my siblings for their assistance, support, care and advice without which I would not have been able to complete this work.

ABSTRACT

Grapes contain bioactive compounds such as polyphenols, resveratrol, flavonoids, and phytoestrogens with antioxidant, anti-inflammatory, and hormone-modulating potentials. Considering the rising prevalence of female infertility and the limitations of conventional therapies, this study explored grapes as a possible natural alternative to enhance reproductive health. This investigation was aimed at evaluating the effect of *Vitis vinifera* (grape) juice on pregnancy outcome in female rats using experimental animal model. Freshly prepared grape juice was administered orally to gravid albino Wistar rats at doses of 2 mL/kg and 5 mL/kg across the three trimesters of pregnancy. Key parameters assessed included maternal weight gain, serum progesterone levels, implantation sites, uterine horn development, pregnancy outcomes, fetal biometric indices, and blood glucose levels. The results showed significant improvements in maternal weight, progesterone concentration, implantation success, litter size, and fetal development in the grape-treated groups compared to controls. Additionally, low-dose grape juice maintained normoglycemia, while higher doses elevated blood glucose, indicating a dose-dependent effect. These findings demonstrate that *Vitis vinifera* juice positively influences maternal physiology, enhances uterine receptivity, and improves pregnancy outcomes, highlighting its potential role as a nutraceutical for supporting female fertility.

CHAPTER ONE

INTRODUCTION

1.0 BACKGROUND OF STUDY

Nutraceuticals is a term that combines "nutrition" and "pharmaceutical" to describe a category of products that blur the line between food and medicine (Durazzo *et al*., 2022). They are substance gotten from food sources, such as plants, animals and microorganisms, and is used to provide medical or health benefits, including the prevention and treatment of diseases. Nutraceutical is a term used commonly with reference to a product derived from a food source for example fruits, vegetables, nuts, spices and herbs that, apart from conferring the basic nutritional value, improve health and wellbeing (Borchers *et al.,* 2016). A nutraceutical is demonstrated to have a Physiological benefit or provide protection against chronic disease. Their bioactive ingredients, the phytochemicals, sustain or promote Health and occur at the intersection of food and pharmaceutical Industries. Such substances may range from isolated nutrients, dietary Supplements and specific diets to genetically engineered designer Foods, herbal products, processed foods and beverages (Kaira, 2003; Prakash *et al.,* 2004).

Nutraceuticals are classified into different classes based principally on their chemical nature or mechanism of action. Therefore, nutraceuticals can include isoprenoid derivates, phenolic compounds, fatty acids, lipid, amino acids, fiber, and carbohydrate molecules capable of exerting specific therapeutic properties, such as antioxidant, anti-inflammatory, antimicrobial, and antineoplastic action (Caponio *et al.,* 2022). Over the past few decades, polyphenols have become a field of interest for nutrition research due to their beneficial health effects. The polyphenols in green and black tea, grapes, and red wine have been intensely investigated.

Grape is a natural source of polyphenols with exceptional biological activities. Most of these polyphenols can be found in grape juice and wine after extraction through pressing and fermentation. However, it is important to note that major parts of biologically active grape polyphenols accumulate in berry seeds and skins, which are disposed with pomace (Georgiev *et al*., 2016).

1.1 JUSTIFICATION OF STUDY

Female infertility is a growing global health concern, affecting millions of women, and it is caused by factors such as oxidative stress, hormonal imbalances, and inflammation. Conventional treatments like hormonal therapy and assisted reproductive technologies are often costly and invasive, creating a need for safer, more affordable alternatives. Grapes, particularly red and purple varieties, are rich in bioactive compounds like resveratrol, polyphenols, and antioxidants, which have shown potential in improving reproductive health by enhancing ovarian function, regulating hormones, and protecting against oxidative damage. While preliminary studies on resveratrol and grape extracts suggest promising effects on fertility, there remains a lack of comprehensive research on how whole grape consumption or supplementation directly influences female reproductive outcomes (Kohut *et al*., 2024). This study aims to investigate the nutraceutical effect of grapes for female fertility.

1.2 AIM

To investigate the nutraceutical effects of Grapes and how its potential effect on fetal development and pregnancy outcome across the three stages of pregnancy.

1.3 Objectives

The specific objectives are to;

1. To evaluate the effects of grape extract administration on the three stages of pregnancy

2. Examine the biological effects on female reproductive physiology

3. To investigate the potential of grape bioactives in enhancing female fertility

CHAPTER TWO

LITERATURE REVIEW

2.0 GRAPES

Grapes are among the most important horticultural crops worldwide. Each year, over 67 million tons are produced. Most of the fruit from about 8 million hectares of vineyards is used to make wine, while the rest is eaten fresh, dried into raisins, made into juice, or distilled into spirits. Archaeological evidence shows that people began cultivating domesticated grapes in the Near East about 6,000 to 8,000 years ago. (McGovern, 2003). Today, there are thousands of grape varieties, created over time through vegetative propagation and crossbreeding. Evidence of ancient winemaking comes from large amounts of tartaric acid and terebinth resin found in clay jars from the late seventh millennium BC. In the Near East, archaeologists have also found many grape seeds from cultivated vines in Chalcolithic and mid Bronze Age sites. (Terral *et al.,* 2010).

During domestication, humans selected for high yield and rapid growth, which led to the loss of resilience factors. In grapevine, changes in berry size, sugar content, and the shift in sexual system from dioecy to self-pollination were essential for domestication. In light of this, it would be relevant to revisit the wild progenitor to identify such factors and make them available for resilience breeding. The resilience factors can be directly incorporated into breeding strategies for preparing European viticulture for the challenges posed by global climate change.

(Grassi and Arroyo, 2020). Grapes are eaten fresh or in dried form, grapes also hold cultural significance for their role in winemaking. Other grape-derived products include various types of jam, juice, vinegar and oil.

2.2 PHYSICAL COMPOSITION OF GRAPES

Grapes are classified as berries, which grow in clusters attached to a stem. Each berry consists of three main parts: the skin, pulp, and seeds. The skin forms the outer layer and is composed of six to ten layers of thick-walled cells. Its surface is coated with a waxy substance known as the cuticle, which makes the berry resistant to water. The skin contains pigments, tannins, aromatic compounds, and minerals such as potassium. Beneath the skin lies the pulp, which occupies most of the berry's volume. Pulp cells have large vacuoles filled with juice, and when the berry is lightly crushed, these delicate cells rupture, releasing what is known as the free-run juice. The seeds are located centrally within the pulp. The berry contains two to four seeds. They are rich in tannin which is extracted during fermentation (Dharmadhikari, 1994).

Plate 2.1: Grapes attached to the stem source: Creasy and creasy 2018.

2.3 CHEMICAL AND PHYTOCHEMICAL COMPOSITION OF GRAPES

Freshly expressed grape juice consists of 70 to 80% water and many dissolved solids. These soluble solids include numerous organic and inorganic compounds. The important group of compounds, from the winemaking point of view, include the following:

2.3.1 SUGARS AND ACIDS

The taste profile of grapes is determined by the balance of sugar and acids and is crucial for winemaking. The cited literature in the broader field of grape chemistry would support the prevalence of glucose, fructose, tartaric, and malic acids (Singh *et al*., 2023).

2.3.2 PHENOLIC COMPOUNDS

Phenolic compounds occur in high concentration in grapes (*Vitis vinifera L.*) and by-products as secondary metabolites responsible for distinct functions associated with plants protection against biological and non-biological environmental stress. The potent antioxidant activity of wine and grape extracts on controlling the oxidation of in vitro low-density lipoproteins were significantly correlated with the action of phenolic compounds present in the samples, especially on the grapes' skin (Barros *et al*., 2015). They are primarily found in the skin, seeds, and to a lesser extent, the pulp. This category includes:

- Flavonoids: Flavonoids are a group of natural substances with different phenolic structures found in fruits, vegetables, grains, bark, roots, stems, flowers, etc Flavonoids, a class of natural compounds recognized for their health-promoting properties, are currently being isolated for further study. They contribute significantly to a wide range of applications in nutraceuticals, pharmaceuticals, medical treatments, and cosmetic formulations due to their antioxidant, anti-inflammatory, anti-mutagenic, and anti-carcinogenic activities, along with their capacity to regulate key cellular enzyme functions. (Panche *et al.,* 2016).
- Flavonols (Quercetin, Kaempferol, Myricetin): These compounds are primarily found in the grape skin and are known for their antioxidant, anti-inflammatory, and potential anti-cancer activities. Quercetin is one of the most abundant flavonols in grapes (Castillo *et al.,* 2011).

2.3.3 STILBENES (RESVERATOL)

Resveratrol, a well-known stilbene is found predominantly in the grape skin. It has garnered significant attention for its potential cardioprotective, anti-cancer, anti-inflammatory, and anti-aging properties (Baur, and Sinclair, 2006).

2.3.4 PHYTOESTROGENS

These are nonsteroidal phytochemicals quite similar in structure and function to gonadal estrogen hormone. They offer an alternative therapy for hormone replacement (HRT) with beneficial effects on cardiovascular system and may even alleviate Menopausal symptoms. They are potential alternatives to the synthetic Selective estrogen receptor modulators (SERMs), which are currently applied in HRT. They have antioxidant effects due to their Polyphenolic nature, anti-carcinogenic, modulation of steroid Metabolism or of detoxification enzymes, interference with calcium transport and favorable effects on lipid and lipoprotein profiles (Prakash and Gupta 2011).

Grapes include different chemicals that are good for the health, such as polyphenol which are most prevalent in red grapes. As well as Nitrogenous compounds, aromatic compounds, Minerals, Pectic Substances (Singh *et al.,* 2023).

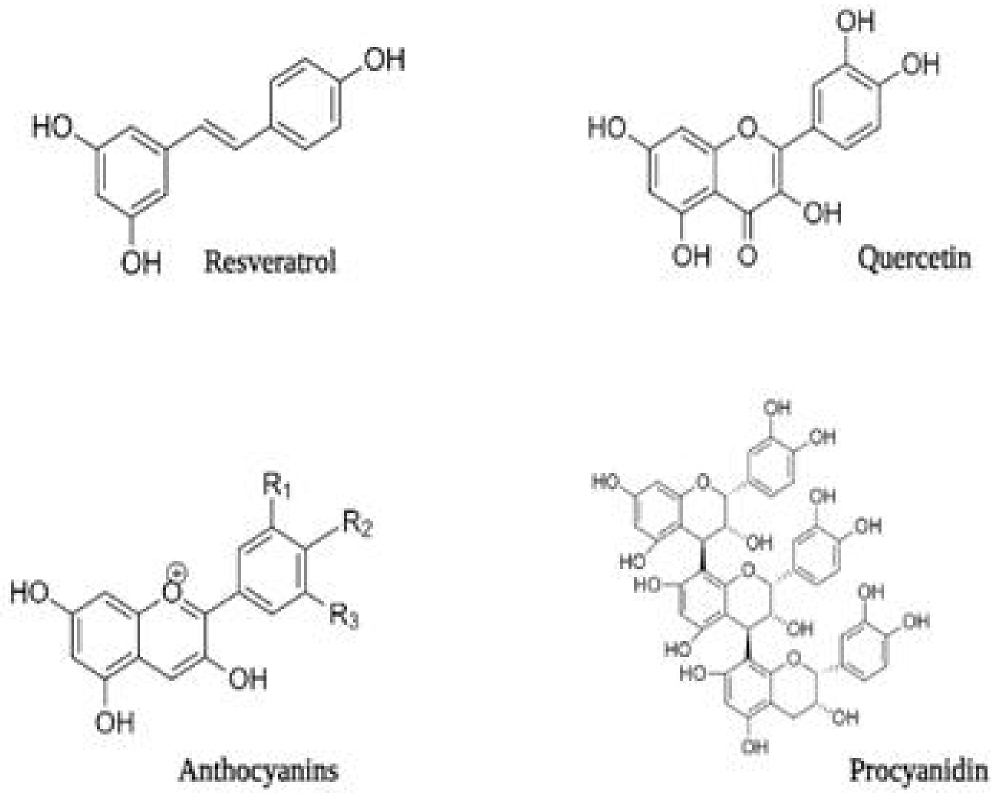

Plate 2.2: Major Phytochemicals found in grape Source Singh *et al.,* 2023.

2.4 VARIETIES OF GRAPES

2.4.1 *Vitis vinifera*

It originates from native to the Mediterranean region. The Grape is thin-skinned. It is used for Wine production, table grapes, raisins. It is widely cultivated globally, with over 99% of the world's grape production (This *et al*., 2006 (Reisch *et al*., 2012).

2.4.2 *Vitis labrusca*

It originates from native to North America. It is thick-skinned. It is used mainly in Juice production, jam, jelly and cultivated in the northeastern United States and Canada. (Reisch *et al*., 2012). They are found in various colors, such as green, red, black, yellow, and pink. Seeded and seedless varieties also exist. (Khan *et al*., 2020).

2.5 NUTRITIONAL COMPOSITION

- **Vitamins:** Grapes contain vitamin C, an antioxidant that supports immune function. They also provide vitamin k which is important for blood clotting and bone health. Vitamin E is also another important natural antioxidant (Aubert and Chalot 2018).
- **Minerals:** Grapes offer minerals like potassium, which helps regulate blood pressure.
- **Potassium:** a mineral that helps to balance fluids in the body, is found in grapes (Whitney and Hammond, 2011).
- **Fiber:** Grapes provide dietary fiber, which promotes digestive health. Dietary fiber, which is found in the skin and pulp of grapes, aids in digestion and helps to maintain regularity (Williams, 2011).
- **Hydration:** Grapes have a high-water content, which can help to keep you hydrated.
- They also contain Carbohydrates, Protein, fiber and other nutrients needed in the body (Rana *et al*., 2022).

2.6 PHARMACEUTICAL PROPERTIES

2.6.1 ANTI-INFLAMMATORY PROPERTIES

A polyphenol found in grapes, resveratrol has been shown to inhibit the production of pro-inflammatory cytokines and enzymes, thereby reducing inflammation (Wang *et al.,* 2018). Also, the anthocyanins present in grapes have been found to possess anti-inflammatory properties, inhibiting the production of inflammatory mediators and reducing oxidative stress (Pandey *et al.,* 2020).

2.6.2 ANTIOXIDANT PROPERTIES

- Polyphenols: Grapes contain a variety of polyphenols, including flavonoids, phenolic acids, and stilbenes, which have been shown to possess antioxidant properties, protecting against oxidative stress and cell damage (Meeran *et al*., 2019).
- Vitamins C and E: Grapes are a good source of vitamins C and E, which are potent antioxidants that help protect cells from oxidative damage (Landrum *et al.,* 2018).

2.6.3 ANTI-CANCER PROPERTIES

Grape is a fruit mainly rich in polyphenols, molecules able to prevent cancer, reduce tumorigenesis, and influence cell proliferation-related pathways (Caponio *et al.,* 2022).

2.6.4 NEUROPROTECTION

The polyphenols particularly the flavanols in grapes maintain cellular protein homeostasis (proteostasis), since impaired proteostasis is closely involved in all amyloid diseases, grape seed extracts may be a valuable therapeutic agent for the prevention and/or management of neurodegenerative diseases (Mahdipour *et al.,* 2022).

2.6.5 ANTIBACTERIAL ACTIVITY

Grape extracts have been demonstrated to exhibit antibacterial properties, inhibiting the growth of certain bacteria. Also, research has also shown that red colored (anthocyanin pigments) grape juice and skin extract have pH-dependent anti-listerial activity. Grape polyphenols may prevent fungal infections (Georgiev *et al.,* 2014).

2.6.6 CARDIOVASCULAR PROPERTIES

Grapes contain flavonoids, which help lower blood pressure and cholesterol levels. The potassium content in grapes helps lower blood pressure, reducing risk of stroke. (Wang *et al*., 2018). Several studies have shown that consumption of grape products may have beneficial effect on cardiovascular system by enhancing endothelial function, decreasing LDL oxidation, improving vascular function, altering blood lipids, and modulating inflammatory process (Li, 2012).

2.6.7 ANTI-DIABETIC EFFECTS AND OBESITY ALLEVIATING PROPERTIES

Grape extracts may regulate blood sugar levels and improve insulin sensitivity. The most common nutrition-related problems in the US are obesity and disorders associated with metabolic syndrome (Chuang and Mclntosh, 2011). By functioning as multi-target modulators with antioxidant and anti-inflammatory properties, polyphenols found in grapes and grape products may lower metabolic syndrome and stop the onset of obesity and type 2 diabetes (Chuang and Mclntosh, 2011). In obese mice, the effects of freeze-dried grape powder and grape powder extracts from red, green, and blue purple seeded and seedless California grapes on inflammation and glucose tolerance were examined (Chuang *et al*., 2012). In obese mice, the authors discovered that grape powder lowers inflammatory markers over time and improves glucose tolerance in the short term (Chuang *et al*., 2012). They also found that quercetin-3-Oglucoside can lower several inflammatory indicators in human adipocytes and was the chemical with the best bioavailability in grape powder extracts (Chuang *et al*., 2012). In addition to its well-established anti-inflammatory and antioxidant properties, an animal model study shown that grape seed extract protects obesity, type 2 diabetes, and metabolic syndrome by enhancing the integrity of the intestinal barrier and regulating metabolic endotoxemia (Goodrich *et al*., 2012).

2.6.8 GASTROPROTECTIVE EFFECTS

Grape polyphenols may protect against gastric ulcers and inflammation.

2.6.9 IMMUNOMODULATORY EFFECTS

Proanthocyanidins extracted from the grape seeds have also been found to have an immune-modulatory role in inflammatory conditions that exert an overproduction of nitric oxide and prostaglandin E2 (Terra *et al.,* 2007).

Plate 3: Pharmaceutical properties of grapes. Source: Usman *et al*., 2023

2.7 THE FEMALE REPRODUCTIVE SYSTEM AND FERTILITY

The female reproductive system comprises internal and external organs that facilitate menstruation and procreation. The internal organs consist of the Vagina, uterus, ovaries and uterine cylinder and the external organs consist of the cervix and the vulva. This organ system is responsible for producing gametes (termed eggs or ova), regulating sex hormones, and maintaining fertilized eggs as they develop into mature fetuses ready for delivery. The Internal female reproductive system has two main parts: the uterus, which houses and nourishes a baby as it grows, and the ovaries, which create the female egg cells. The female reproductive system is structured in a way that allows it to carry out several functions. It does so by producing the egg cells necessary for reproduction, transporting the eggs to the site of fertilization which is the fallopian tube. The next step is for the embryo to attach itself to the uterine wall and begin the step for pregnancy. In addition to this the female reproductive system is also responsible for generation of sexual hormones which help with the continuation of the reproductive cycle. A woman can get pregnant from the time she starts her period which is the first menstrual cycle until she hasn't had one for 12 months in a row when she reaches menopause. Fertility is the ability to conceive and produce children (Barbieri, 2019).

Pregnancy is the period of time during which the foetus grows or develops in the uterus. The embryo develops into a foetus. Gestation period is usually 40 weeks in humans, while in rats it is about 3weeks (21-23 days). For rats there are three trimesters:

1st Trimester: The implantation stage

2nd Trimester: Organogenesis and fetal growth

3rd Trimester: Preparation for birth

The inability to conceive after a year (12 months) of consistent, unprotected sexual activity is known as infertility. Those who have never conceived are classified as having primary infertility, whereas those who have conceived in the past but are currently experiencing infertility are classified as having secondary infertility. Infertility may result from male, female, or both reasons. Ovulatory disorders, endometriosis, pelvic adhesions, tubal obstruction, various uterine/tubal abnormalities, and hyperprolactinemia are the most prevalent female variables that contribute to infertility, in decreasing order (Walker and Tobler, 2022).

Infertility can be caused by a variety of illnesses. Most infertility instances are caused by other illnesses. These conditions may harm the fallopian tubes, disrupt ovulation, or result in hormonal issues. Among the primary health issues linked to infertility are Up to 90% of ovulation cases are caused by polycystic ovarian syndrome (PCOS), which is typically a genetic condition (Barbier, 2001). Ovulation issues, tubal obstruction, age-related variables, uterine issues, hormone imbalance, and the decisions made by the modern lifestyle—such as the higher average age of marriage, stress, the unfavorable legislative framework for assisted reproduction, etc can also result in infertility (Roupa *et al*., 2009).

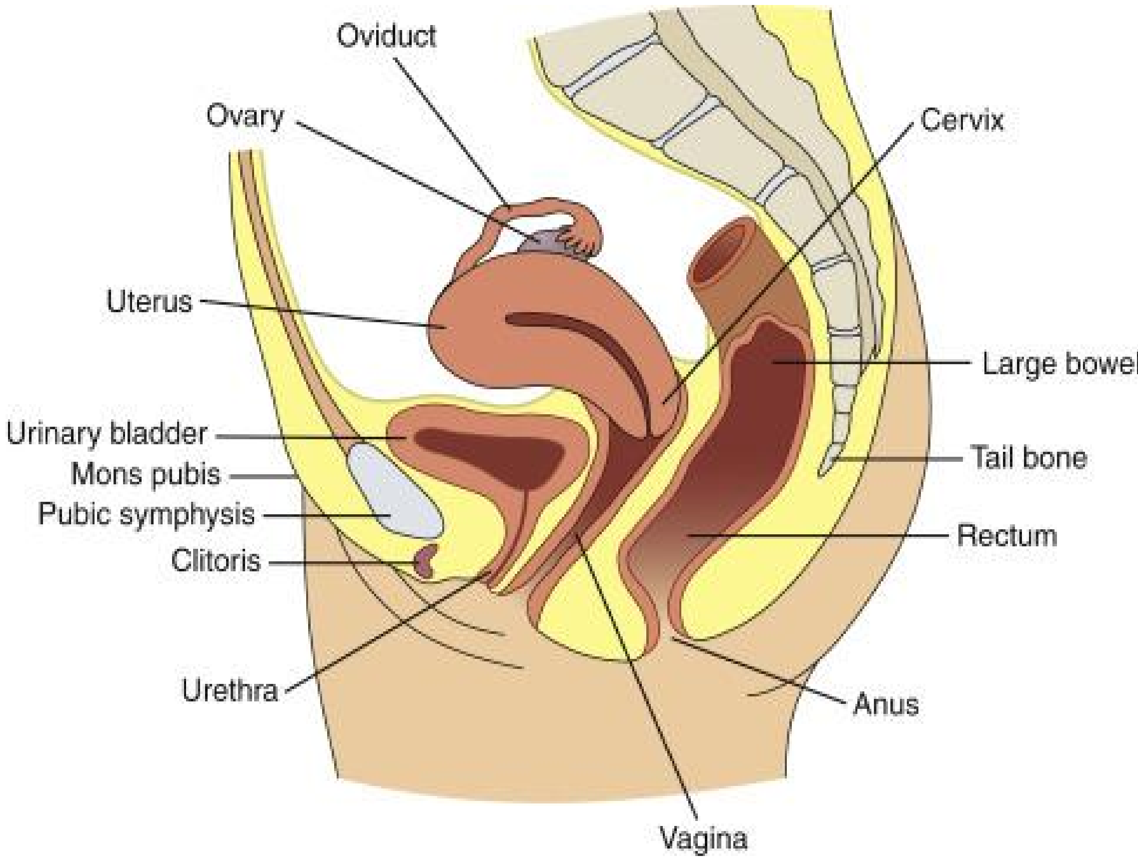

Figure 2.3: Side view of the female pelvic region showing some major components of the reproductive system. (Richard and Kristin, 2014).

2.8 FERTILITY DRUGS

2.8.1 CLOMIPHENE CITRATE

Clomiphene Citrate (CC) is a selective estrogen receptor modulator (SERM), with estrogen receptor agonist and antagonist properties. Estrogen agonist actions occur at low endogenous estrogen concentrations, otherwise CC acts mainly as a competitive antagonist at the estrogen receptor (Melmed *et al*., 2011). CC is a racemic mixture of the two stereoisomers enclomiphene and zuclomiphene, with the former being the stereoisomer mainly responsible for its efficacy in ovulation induction (Bhagavath and Carlson, 2012). CC is metabolized in the liver and excreted in the stool, with a relatively long half-life of 5 to 7 days. The less potent stereoisomer zuclomiphene remains detectable for much longer period, without major clinical relevance (Christou *et al*., 2017). At the level of the hypothalamus, CC binds and depletes estrogen receptors. This leads to an inhibition of the negative feedback effect of circulating estradiol, which in turn increases the pulse frequency of hypothalamic GnRH. This results in increased LH and FSH production by the pituitary gland. In women with polycystic ovary syndrome, CC is highly effective in inducing ovulation and was until recently recommended as the initial treatment of choice for most anovulatory or oligo-ovulatory infertile women (Bhagavath and Carlson, 2012), despite some data regarding potential detrimental effects on cervical mucus and endometrial thickness (Gadalla *et al*., 2018) of debatable clinical impact. A randomized multicenter trial comparing CC, the insulin sensitizer metformin, and both medications combined in 626 patients with the polycystic ovary syndrome found live-birth rates of 22.5%, 7.2% and 26.8% in the clomiphene group, metformin group, combination-therapy group, respectively.

Among pregnancies, the rate of multiple pregnancy was 6.0% in the clomiphene group, 0% in the metformin group, and 3.1% in the combination-therapy group (Legro *et al*., 2014).

2.8.2 LETROZOLE

Aromatase, a member of the cytochrome P450 superfamily, is the enzyme catalyzing the ratelimiting step in the biosynthesis of estrogens. Also termed CYP19A1, aromatase is responsible for the aromatization of androgens into estrogens. Aromatase inhibitors are used in oncology as adjuvant therapy for postmenopausal women with breast cancer. Given that aromatase inhibitors dramatically lower systemic estrogen concentrations, investigators attempted to use them for ovulation induction by preventing estrogen negative feedback on FSH (Casper and Mitwally, 2012). Initial pilot studies demonstrated high rates of ovulation induction in patients with polycystic ovary syndrome using the aromatase inhibitor letrozole, with few side effects. However, results from an abstract at the 2005 American Society of Reproductive Medicine (ASRM) meeting suggested an increased rate of cardiac malformations in babies born to women who underwent fertility treatment involving letrozole (Casper and Mitwally, 2012). The abstract was never published, but it prompted the manufacturer of letrozole to issue a "black box warning" on its use in women with premenopausal endocrine status, as well as in lactation and pregnancy. Subsequent published research of higher quality than the 2005 abstract demonstrated that the incidence of congenital cardiac anomalies in babies born to mothers using letrozole is significantly lower than the one in babies born to mothers using CC, and lower than in the general population (Tatsumi *et al*., 2017).

In 2014, 750 women with polycystic ovary syndrome according to modified Rotterdam criteria were randomly assigned to receive letrozole or clomiphene for up to five treatment cycles in a double-blind, multicenter trial (Legro *et al*., 2014). The cumulative live birth rate was 27.5% in the letrozole arm versus 19.1% in the clomiphene arm (P ¼ 0.007); rate ratio for live birth,1.44; 95% confidence interval,1.10 to 1.87 (Legro *et al*., 2014). There was no significant difference in the rates of overall congenital anomalies, pregnancy loss or twin pregnancy. Clomiphene was associated with a higher incidence of hot flushes, and letrozole was associated with higher incidences of fatigue and dizziness.

2.8.3 FOLIC ACID

Folic acid, sometimes referred to as folate or vitamin B9, is a B vitamin and a necessary cofactor for enzymes that synthesize DNA and RNA. The body needs folic acid to synthesize purines, pyrimidines, and methionine before incorporating them into DNA or proteins.

Foods-like liver, kidney, yeast, and leafy green vegetables contain folic acid, a water-soluble B-complex vitamin. Folic acid, sometimes referred to as vitamin B9, is a necessary cofactor for enzymes that are involved in DNA and RNA synthesis. More precisely, before being incorporated into DNA or proteins, the body needs folic acid for the synthesis of purines, pyrimidines, and methionine.

In addition to preventing neural tube defects, folic acid may also prevent the following congenital abnormalities and low birthweight: cardiovascular disease, cerebral stroke, cancer of multiple sites, depression, dementia, osteoporosis, and others. The enzyme dihydrofolate reductase, which is dependent on nicotinamide adenine dinucleotide phosphate hydrogen (NADPH), transforms the synthetic form of folic acid, known as dihydrofolate (DHF), into THF. After that, THF transforms into 5-10-methylenetetrahydrofolate (5-10-MTHF), which can go in one of two directions: either toward methionine synthesis or DNA synthesis using dTMP (Lan *et al*., 2018). The World Health Organization (WHO) advises all women trying to conceive to take

0.4 mg of folic acid per day as a supplement (World Health Organization, 2017). Due to the possibility of neural tube closure before many women are aware of their pregnancy, supplements are advised prior to conception rather than after pregnancy confirmation (Toivonen *et al.*, 2018).

2.9 EFFECT OF GRAPES ON FEMALE REPRODUCTIVE SYSTEM AND FERTILITY

2.9.1 ON REPRODUCTIVE PROCESSES

Muscle growth and reproductive efficacy are negatively correlated, which may indicate reproductive dysfunctions. Oxidative stress can cause inflammation, which can then trigger several reproductive disorders, such as ovarian cancer or various reproductive defects, including endometriosis, polycystic ovary syndrome (PCOS), oocyte mutation, and ovarian folliculogenesis. It can also induce oocyte maturation and the release of sex hormones (Predescu *et al*., 2019; Wang *et al*., 2020). The anti-inflammatory and antioxidant capabilities that bioactive phytonutrients are known to impart may have a positive effect on reproductive processes (Atanasov *et al*., 2015; Forni *et al*., 2019). The physiological functioning of reproductive organs may be impacted by polyphenols' ability to penetrate different protective barriers (Wocławek-Potocka *et al*., 2013).

2.9.2 EFFECT ON THE OVARIES

Oxidative stress is a significant factor in ovarian aging and can cause both human and animal fertility to diminish (Liu *et al*., 2018). Shen *et al*. (2012) state that oxidative stress-induced apoptotic events in granulosa cells are thought to be a primary cause of follicular atresia. After treating oocytes, polyphenols have been shown to increase the quantity and quality of oocytes in both people and animals (Sun *et al*., 2019). According to the mean numbers of cleavage, morula, and blastocyst rates, sheep have shown positive effects of grapeseed extract on oocyte maturation and early development. Mice's oocyte viability can be positively impacted by grapeseed procyanidin B2, which can also enhance their maturation and developmental potential (Luo *et al*., 2020). Additionally, grapeseed extract may be useful in treating or preventing PCOS. The metabolic state of PCOS-positive women improved with short-term grapeseed extract treatment. Additionally, grapeseed extract can prevent undesirable morphological changes in the ovaries caused by reproductive aging and have a positive effect on health in cases of menopause and reproductive insufficiency (Lin *et al*., 2018). Proanthocyanidin B2, which has been seen in rat ovaries as a potential defense against age-dependent degenerative alterations, may be the cause of this action (Lin *et al*., 2018). Proanthocyanidin B2 from grapeseed has been shown in several studies to protect rat ovarian tissue from ischemia or ischemia/reperfusion-induced injury (Zhang *et al*., 2016; Lin *et al*., 2018).

Grapeseed extract can affect resistance to chemotherapy and reduce human ovarian cancer cell growth (Zhao *et al*., 2013). Delphinidin such as a member of the anthocyanidin family and a natural pigment in grapes may be a pivotal therapeutic target for the prevention of epithelial ovarian cancer (Lim and Song, 2017). Grapeseed extract, as well as proanthocyanidin B2 can modulate human granulosa cell functions, including steroidogenesis, and can exert phytoestrogenic activity with a positive effect on steroid hormone production in human granulosa cells (Barbe *et al*., 2019).

2.9.3 EFFECT ON UTERUS

The potential effects of grapeseed extract on endometrial functions were discussed by (Colitti *et al*. 2007). The oral treatment of grapeseed extract in heifers influenced the expression of several genes in the uterine endometrium. Additionally, endometriosis may be avoided because to the anti-inflammatory qualities of resveratrol, which is found in grapes. In the prevention and/or treatment of endometriosis, this well-known phytonutrient has been regarded as a novel medication (Bruner-Tran *et al*., 2011). Additionally, resveratrol has been shown to reinforce hormone action during human endometrial stromal cell (ESC) development and modify the endometrium's responsiveness to progesterone and estrogen during decidualization, all of which may benefit women's health (Citrinovitz *et al*., 2020). Proanthocyanidins in grapeseeds have been shown in another investigation to have promising chemopreventive qualities against cervical cancer. Through the mitochondrial signaling route, proanthocyanidin B2 can cause apoptosis and inhibit the growth and proliferation of cervical cancer. Accordingly, the research that is now available points to the effects of grape polyphenols on decidualization, the uterine endometrium, and their potential to prevent and/or treat cervical cancer and endometriosis.

2.9.4 EFFECTS ON REPRODUCTIVE HORMONES

The essential regulators of reproductive processes, such as steroid hormones (estradiol, progesterone, testosterone), prostaglandins, and hypothalamic neurohormones (GnRH, oxytocin, LH, and FSH), can be impacted by phenolic chemicals found in grapes (Hashem *et al*., 2020). Furthermore, by binding or activating estrogen receptors (ERα and ERß), polyphenols may have hormone-like effects (either estrogen-agonistic or antagonistic) because of their chemical resemblance to the structure of estrogens. Red wine contains a flavonol called myricetin, which can inhibit the production of progesterone by granulosa cells triggered by insulin-like growth factor I (IGF-I) and promote the creation of estradiol by IGF-I (Spicer and Schütz, 2022). Similarly, resveratrol can result in increased decidualization of human embryonic stem cells (ESCs) in vitro by increasing the production of prolactin and IGF-I binding protein 1 (IGFBP1). By boosting the expression and activation of insulin receptors, grapeseed extract can affect insulin sensitivity (Meeprom *et al*., 2011).

CHAPTER THREE

3.1 MATERIALS AND METHODS

3.1. Collection of Plant sample and Preparation of *Vitis vernifera* juice

The *Vitis vernifera* was purchased in March 2025 from Ovbiogie Market in Ovia South, Benin City, Nigeria. The seedless fruit of *Vitis vernifera* was washed and juiced with a food processor freshly prepared daily and kept in an airtight container for subsequent usage.

3.2 Preparation of stock solution

Daily administration of 2 and 5 mls/kg was calculated on the basis of the weights of the gravid dams and doses that were precisely calculated and measured to be administered to the animals during the experiment (Oshomoh and Obaro-Onezeyi, 2019).

3.3 Chemicals

Chloroform (supplied by Fharmatrends Nigeria Ltd), Sodium Chloride all of analytical standards.

3.4 Drugs

Folic acid, oxytocin, were of pure samples and pharmaceutical standards.

3.5 Experimental Design

3.5.1 Acute Toxicity Study

Methods which was used to conduct a study on acute toxicity is OECD (Organization of economic co-operation development), 2008a guidelines. Six (6) female mice were administered 10 mls/kg per body weight of the extract and observed for 72 hours for possible signs of toxicity, mortality or morbidity.

3.6 Experimental animals

A total of 25 adult albino Wistar rats consisting of 5 males and 20 females with average weight of 200-280 grams were gotten through the animal house of the Phytomedicine unit in the Department of Plant Biology and Biotechnology, University of Benin. The animals were kept in wooden cages with ambient temperature and kept in typical laboratory circumstances, which include 12-hour cycles of light and darkness. The rats were provided standard pelletized layers mash and clean water for two weeks as acclimatization period prior to the experimental study.

3.7 Grouping and mating of animals

The weights of the female Wistar rats were measured using an electronic weighing balance. The rats were grouped into five (5): I, II, III, IV and V of 5 rats each for the first, second and third trimesters of pregnancy. Male Wistar rats obtained were introduced into each group for mating in a ratio 1:1. Only female rats at pro-estrus after microscopic (UIS450, USA) examination were mated. The female rats were left overnight with their male counterparts.

3.8 Examination of vaginal smear

The next morning after mating, vaginal smear was taken from each female rat in each group and observed by microscopic examination for the presence of sperm. Pipette containing little distilled water was inserted into the vagina of each rat and the vagina fluid was taken, smeared on the glass slides, covered with cover slips and viewed under the light microscope. The presence of sperm cells in the vaginal fluid confirmed a positive sperm test and also a successful mating and was thereby taken as day 0 of pregnancy according to Obaro and Oshomoh (2019).

3.9 Administration of extract

Extract was freshly prepared every morning and administered orally to rats by carefully inserting an orogastric tube into the oral cavity of the rats. The animals were grouped into three trimester groups; first trimester (^{a}), second trimester (^{b}) and third trimester (^{c}), divided into 3 sub-groups (Group I^{a-c}, II^{a-c}, III^{a-c}, IV^{a-c} and V^{a-c}) consisting of 5 animals each.

Group I (control) – Normal saline (2 ml/kg)

Group II- Positive control (5 mg/kg folic acid)

Group III- Negative control (10 IU of oxytocin)

Group IV and V (2 ml/kg of the extract respectively after acute toxicity study)

Group IV and V (5 ml/kg of the extract respectively after acute toxicity study).

Throughout the period of administration, food and water were given to the rats. For the first trimester group, the extracts were administered to the pregnant dams from day 0 - 7 of pregnancy and the rats were sacrificed on the 8th day of pregnancy.

For the second trimester group, the extracts were administered from day 0 - 14 of pregnancy and the rats were sacrificed on the 15th day of pregnancy.

For the third trimester group, the extracts were administered from day 0 - 21 and the rats were left to litter.

The weights of the rats in the control as well as treatment groups were recorded on day 0, 7, 14 and day 21 of the gestation period. Examination were carried out every day to look for toxicity indicators which include; salivation, shedding tears, writhing, convulsion, tremors, yellow coloration of fur, hair loss, bleeding, and/or mortality.

3.10 Determination of serum progesterone level

Blood samples was withdrawn from the animals on day 8 and 15 of pregnancy via cardiac puncture, centrifuged at 3000 revolutions per minute for 10 minutes and serum obtained was analyzed using an Enzyme linked immunosorbent assay (ELISA) kit to evaluate serum progesterone levels according to Oshomoh and Obaro (2020).

3.11 Pregnancy outcome

On the 8th and 15th day of gestation, the female rats were laparotomized under chloroform anesthesia. The lower abdomen was cut open and the uterus was examined for number of implantation sites and width of uterine horns on day 8 and day 15 of pregnancy respectively (Oshomoh and Obaro, 2019).

3.12 Parturition

After parturition, the number of alive and still born pups was recorded. All puppies were examined for obvious outward abnormalities including open eyelids and aberrant tails and club foot. The birth weight, gestation length, litter size, crown-rump length and tail length of the pups were recorded according to Oshomoh and Obaro (2019).

3.13 Statistical analysis

Every values presented as Mean ± Standard Error of Mean (SEM). Using the UK's Graph Pad Prism 8.2 software, one-way ANOVA was used to analyze the data. $P \leq 0.05$ was used to define significance for differences.

CHAPTER FOUR

RESULTS

4.1 Effect **of *Vitis vernifera* (VVJ) on body weight of gravid dams in 1st, 2nd and 3rd trimesters.**

4.2 Effect of *Vitis vernifera* (VVJ) on Serum level of Progesterone in Female Rats at First and Second Trimesters of pregnancy.

Figure 4.2: Serum level of progesterone in pregnant rats administered 2 and 5 mls/kg of *Vitis vernifera Juice* (VVJ) *= (*P≤ 0.05*) $^{\alpha}$ = (*P≤0.01*) shows a substantial difference from the control.

Figure 4.1: Effect of Aqeous extract of *Vitis vernifera* juice (VVJ) on body weight of gravid dams at first, second and third trimester of pregnancy.

Values are expressed in mean ± SEM in contrast to the control, n=5. *= *P*≤ 0.05; α= *P≤ 0.01*; β= *P≤ 0.001*

Plate 4.1 A, B C, D and E: Photograph of the uterus of pregnant Wistar rats at 1st trimester (8th day) of pregnancy.

Key:

- A= Normal Saline (2 mi/kg) (Control)
- B= Clomiphene citrate (10 mg/kg)
- C= Oxytocin (10 IU)
- D= Aqeous extract of ***Vitis vernifera*** **Juice (VVJ)** 2 mls/kg
- E= Aqeous extract of ***Vitis vernifera*** **Juice** **(VVJ)** 5 mls /kg
- RU= Right Uterine horn
- LU= Left Uterine horn.
- IS= Implantation Site
- NIS= No Implantation Site

4.3 Effect of *Vitis vernifera* Juice (VVJ) on implantation and width of uterine horns gravidity of pregnant dams at first, second trimesters.

Figure 4.3: Effect of ***Vitis vernifera*** **(VVJ)**) on implantation and width of uterine horns gravidity of pregnant dams at first trimester.

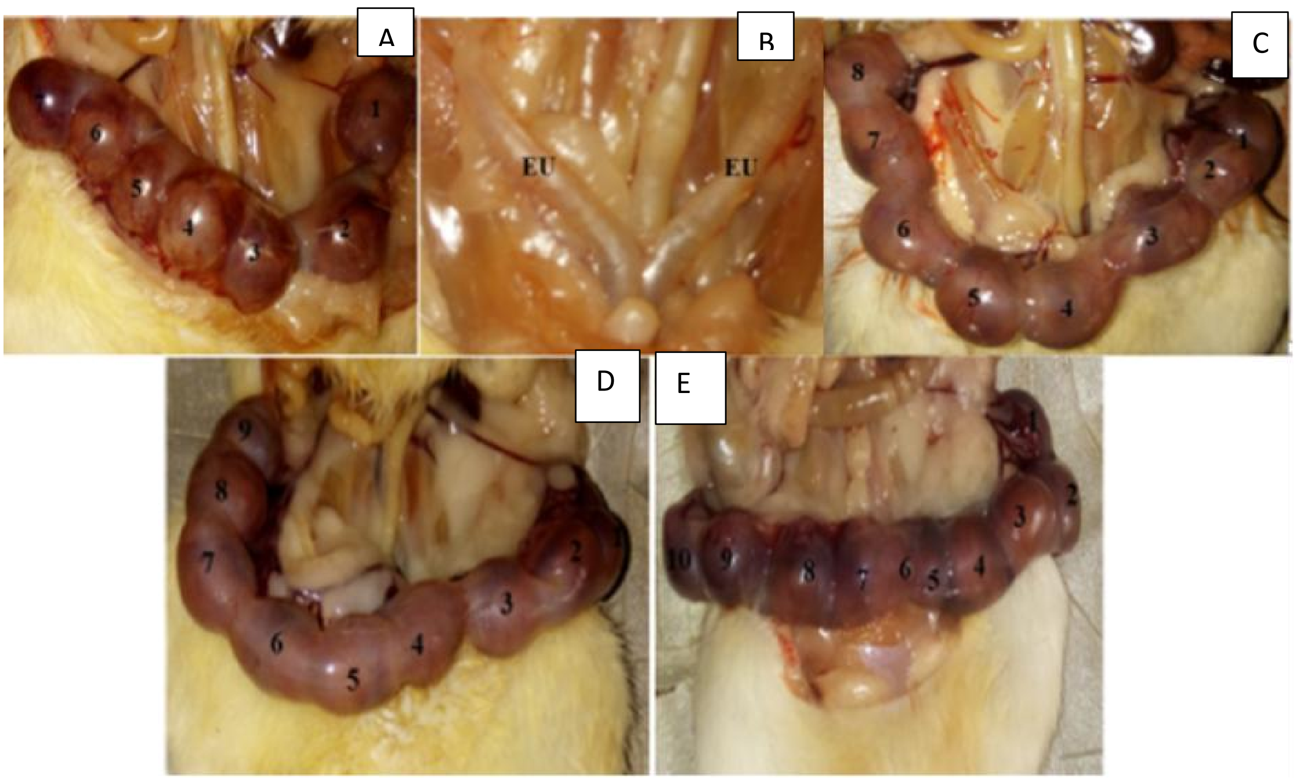

Plate 4.2 A, B C, D and E: Photograph of the uterus of pregnant Wistar rats at 2nd trimester (15th day) of pregnancy.

Key:

A= Control

B= Clomiphene citrate (10 mg/kg)

C= Oxytocin (10 IU)
D= ***Vitis vernifera*** **(VVJ)** 2 mls /kg
E= ***Vitis vernifera*** **(VVJ)** 5 mls /kg
EU= Empty Uterus

4.4 Effect of *Vitis vernifera* Juice (VVJ)) in pups after delivery

Figure 4.4: Effect of ***Vitis vernifera*** Juice **(VVJ)** *on* implantation and width of uterine horns

Figure 4.5: Effect of Aqeous Extract of ***Vitis vernifera*** **juice (VVJ)** on Some Parameters of Pups Born to pregnant dams at after 3rd trimester Parturition.

Table 4.6: The effects of *Vitis vernifera* on fasting blood glucose Level

CHAPTER FIVE

5.0 DISCUSSION

5.1 MATERNAL HEALTH AND WEIGHT GAIN

5.2 EFFECTS ON PROGESTERONE LEVEL

5.3 EFFECTS ON IMPLANTATION

5.4 EFFECTS ON PREGNANCY OUTCOMES AND FETAL PARAMETERS

5.5 EFFECT ON BLOOD GLUCOSE LEVEL

CONCLUSION

Vitis vinifera juice (VVJ) possesses significant nutraceutical properties that exert a positive multi-targeted influence on female reproductive health and fertility outcomes. The administration of VVJ enhanced maternal nutritional status, significantly increased serum progesterone levels, improved uterine receptivity as evidenced by a greater number of implantation sites, and then led to superior pregnancy outcomes with an increase in live pup numbers and improved fetal biometric parameters.

REFERENCES

Atanasov, A. G., Waltenberger, B., Pferschy-Wenzig, E. M., Linder, T., Wawrosch, C., Uhrin, P., Temml, V., Wang, L., Schwaiger, S., Heiss, E. H. and Rollinger, J. M. (2015). Discovery and resupply of pharmacologically active plant-derived natural products: A review. *Biotechnology Advances*, **33**(8): 1582-1614.

Aubert, C. and Chalot, G. (2018). Chemical composition, bioactive compounds, and volatiles of six table grape varieties (*Vitis vinifera L.*). *Food Chemistry*, **240**: 524-533.

Barbe, A., Ramé, C., Mellouk, N., Estienne, A., Bongrani, A., Brossaud, A., Riva, A., Guérif, F., Froment, P. and Dupont, J. (2019). Effects of grape seed extract and proanthocyanidin B2 on in vitro proliferation, viability, steroidogenesis, oxidative stress, and cell signaling in human granulosa cells. *International Journal of Molecular Sciences*, **20**(17): 4215.

Barros, A., Gironés-Vilaplana, A., Texeira, A., Baenas, N. and Domínguez-Perles, R. (2015). Grape stems as a source of bioactive compounds: application towards added-value commodities and significance for human health. *Phytochemistry reviews*, **14**, 921-931.

Baur, J. A. and Sinclair, D. A. (2006). Therapeutic potential of resveratrol: the in vivo evidence. *Nature Reviews Drug Discovery*, **5**(6), 493-506.

Bhagavath, B. and Carson, S. A. (2012). Ovulation induction in women with polycystic ovary syndrome: an update. *American Journal of Obstetrics and Gynecology*, **206**(3): 195-198.

Borchers, A. T., Keen, C. L., Gershwin, M. E. (2016) The basis of structure/function claims of nutraceuticals. *Clinical Review and Allergy Immunology*, **51**, 370–382.

Caponio, G. R., Lippolis, T., Tutino, V., Gigante, I., De Nunzio, V., Milella, R. A., Gasparro, M., and Notarnicola, M. (2022). Nutraceuticals: Focus on Anti-Inflammatory, Anti-Cancer, Antioxidant Properties in Gastrointestinal Tract. Antioxidants. **11**(7): 1274.

Casper, R. F. and Mitwally, M. F. (2012). A historical perspective of aromatase inhibitors for ovulation induction. *Fertility and sterility*, **98**(6): 1352-1355.

Chuang, C. C. and McIntosh, M. K. (2011). Potential mechanisms by which polyphenol-rich grapes prevent obesity-mediated inflammation and metabolic diseases. *Annual Review of Nutrition*, **31**(1): 155-176.

Chuang, C. C., Shen, W., Chen, H., Xie, G., Jia, W., Chung, S. and McIntosh, M. K. (2012). Differential effects of grape powder and its extract on glucose tolerance and chronic inflammation in high-fat-fed obese mice. *Journal of Agricultural and Food Chemistry*, **60**(51): 12458-12468.

Christou, F., Pitteloud, N. and Gomez, F. (2017). The induction of ovulation by pulsatile administration of GnRH: an appropriate method in hypothalamic amenorrhea. *Gynecological Endocrinology*, **33**(8): 98-601.

Citrinovitz A. C. M, Langer L, Strowitzki T, and Germeyer A. (2020). Resveratrol enhances decidualization of human endometrial stromal cells. *Reproduction*, **159**(4):453–463.

Colitti, M., Sgorlon, S., Stradaioli, G., Farinacci, M., Gabai, G. and Stefanon, B. (2007). Grape polyphenols affect mRNA expression of PGHS-2, TIS11b and FOXO3 in endometrium of heifers under ACTH-induced stress. *Theriogenology*, **68**(7): 1022-1030.

Dharmadhikari, M., 1994. Composition of grapes. *Vineyard Vintage View Mo State Univ*, **9**(7/8): 3-8.

Durazzo, A., Lucarini, M. and Santini, A. (2020). Nutraceuticals in human health. *Foods*, **9**(3): 370.

Forni, C., Facchiano, F., Bartoli, M., Pieretti, S., Facchiano, A., D'Arcangelo, D., Norelli, S., Valle, G., Nisini, R., Beninati, S. and Tabolacci, C., 2019. Beneficial role of phytochemicals on oxidative stress and age-related diseases. *BioMed research international*, **2019**(1): 8748253.

Gadalla, M. A., Huang, S., Wang, R., Norman, R.J., Abdullah, S. A., El Saman, A. M., Ismail, A. M., van Wely, M. and Mol, B. W. (2018). Effect of clomiphene citrate on endometrial thickness, ovulation, pregnancy and live birth in anovulatory women: systematic review and meta-analysis. *Ultrasound in Obstetrics & Gynecology*, **51**(1): 64-76.

Georgiev, V., Ananga, A. and Tsolova, V. (2016). Dietary supplements/nutraceuticals made from grapes and wines. *Wine safety, consumer preference, and human health*, 201-227.

Georgiev, V., Ananga, A. and Tsolova, V. (2014). Recent advances and uses of grape flavonoids as nutraceuticals. *Nutrients*, **6**(1), 391-415.

Goodrich, K. M., Fundaro, G., Griffin, L. E., Grant, A.Q., Hulver, M. W., Ponder, M. A. and Neilson, A. P. (2012). Chronic administration of dietary grape seed extract increases colonic expression of gut tight junction protein occludin and reduces fecal calprotectin: a secondary analysis of healthy Wistar Furth rats. *Nutrition Research*, **32**(10): 787-794.

Grassi Fabrizio. and Arroyo-Garcia Rosa. 2020. Editorial: Origins and Domestication of the Grape. *Frontiers in Plant Science*, **11**: 1664-462.

Hashem, N. M., Gonzalez-Bulnes, A. and Simal-Gandara, J. (2020). Polyphenols in farm animals: source of reproductive gain or waste. *Antioxidants*, **9**(10): 1023.

Kalra, E. K.(2003) Nutraceutical: Definition and Introduction. *AAPS PharmSciTech*, **5**: 1-2.

Khan, S. S., Singh, S.K and Sharma, H. C (2020). Seedless grape breeding: A review. *Journal of horticultural Science and Biotechnology*, **95**(3): 257-265.

K.I. Toivonen, E. Lacroix, M. Flynn, P.E. Ronksley, K.A. Oinonen, A. Metcalfe, T.S. Campbell. (2018). Folic acid supplementation during the preconception period: A systematic review and meta-analysis.*Preventive Medicine*,**114** 1-17.

Kohut, L., Baldovska, S., Mihal, M., Belej, L., Sirotkin, A. V., Roychoudhury, S., and Kolesarova, A. (2024). The multiple actions of grape and its polyphenols on female reproductive processes with an emphasis on cell signalling. *Frontiers in Endocrinology*, **14**: 1245512.

Legro, R. S., Brzyski, R. G., Diamond, M. P., Coutifaris, C., Schlaff, W. D., Casson, P., Christman, G. M., Huang, H., Yan, Q., Alvero, R. and Haisenleder, D. J. (2014). Letrozole versus clomiphene for infertility in the polycystic ovary syndrome. *New England Journal of Medicine*, **371**(2): 119-129.

Li, H. and Förstermann, U. (2012). Red wine and cardiovascular health. *Circulation Research*, **111**(8): 959-961.

Lim, W. and Song, G. (2017). Inhibitory effects of delphinidin on the proliferation of ovarian cancer cells via PI3K/AKT and ERK 1/2 MAPK signal transduction. *Oncology Letters*, **14**(1): 810-818.

Liu, X., Lin, X., Mi, Y., Li, J. and Zhang, C. (2018). Grape seed proanthocyanidin extract prevents ovarian aging by inhibiting oxidative stress in the hens. *Oxidative Medicine and Cellular Longevity*, **2018**(1): 9390810.

Luo, Y., Zhuan, Q., Li, J., Du, X., Huang, Z., Hou, Y. and Fu, X. (2020). Procyanidin B2 improves oocyte maturation and subsequent development in type 1 diabetic mice by promoting mitochondrial function. *Reproductive Sciences*, **27**: 2211-2222.

Mahdipour, R., Ebrahimzadeh-Bideskan, A., Hosseini, M., Shahba, S., Lombardi, G., Malvandi, A. M. and Mohammadipour, A. (2023). The benefits of grape seed extract in neurological disorders and brain aging. *Nutritional Neuroscience*, **26**(5): 369-383.

Meeprom, A., Sompong, W., Suwannaphet, W., Yibchok-anun, S. and Adisakwattana, S. (2011). Grape seed extract supplementation prevents high-fructose diet-induced insulin resistance in rats by improving insulin and adiponectin signalling pathways. *British Journal of Nutrition*, **106**(8): 1173-1181.

Majeed, U., Shafi, A., Majeed, H., Akram, K., Liu, X., Ye, J. and Luo, Y., 2023. Grape (*Vitis vinifera L.*) phytochemicals and their biochemical protective mechanisms against leading pathologies. *Food Chemistry*, **405**: 134762.

Melmed, S., Casanueva, F. F., Hoffman, A. R., Kleinberg, D. L., Montori, V. M., Schlechte, J. A. and Wass, J. A. (2011). Diagnosis and treatment of hyperprolactinemia: an Endocrine Society clinical practice guideline. *The Journal of Clinical Endocrinology and Metabolism*, **96**(2): 273-288.

Morabito, N., Crisafulli, A., Vergara, C., Gaudio, A., Lasco, A., Frisina, N., D'Anna, R., Corrado, F., Pizzoleo, M.A., Cincotta, M. and Altavilla, D. (2002). Effects of genistein and hormone-replacement therapy on bone loss in early postmenopausal women: a randomized double-blind placebo-controlled study. *Journal of Bone and Mineral Research*, **17**(10): 1904-1912.

Obaro-Onezeyi O.E and Emmanuel Oshomoh (2019) Assessment of several concentration of Aqueous extract of *Croton* lobactus on reproductive parameters. *Nigerian Journal of Science and Technology Research*.1 (1); 72-80.

Oshomoh Emmanuel and Obaro-Onezeyi O.E (2020) Effect of Aqueous extract of Talinium triangulare on reproductive parameters of female Wistar rats. *ACTA Scientific* 4: 02-10.

Panche, A. N., Diwan, A. D. and Chandra, S. R. (2016). Flavonoids: an overview. *Journal of Nutritional Science*, **5**: e47.

P. E. McGovern, U. Hartung, V. R. Badler, D. L. Glusker, L. J. Exner. (1997). The beginnings of winemaking and viniculture in the ancient Near East and Egypt. *Expedition* **39**, 3–21.

Prakash, D., Dhakarey, R. and Mishra, A. (2004). Carotenoids: The Phytochemicals of nutraceutical importance. *Indian Journal of Agricultural Biochemistry,* **17**: 1-8.

Prakash, D. and Gupta C. (2011). Role of Phytoestroges as Nutraceuticals in human health. *Pharmacologyonline*, **1**: 510-523.

Predescu, D. V., Crețoiu, S. M., Crețoiu, D., Alexandra Pavelescu, L., Suciu, N., Radu, B. M. and Voinea, S. C. (2019). G protein-coupled receptors (GPCRs)-mediated calcium signaling in ovarian cancer: Focus on GPCRs activated by neurotransmitters and inflammation-associated molecules. *International Journal of Molecular Sciences*, **20**(22): 5568.

Rana, A., Kaur, J., Sharma, K., Singh, J., and Bhadariya, V. (2022). A comprehensive review on nutritional value and health benefits of grape leaves. *PharmaInnovation Journal*. 2235-2243.

Richard E. Jones, Kristin H. Lopez. (2014). The Female Reproductive System. Human Reproductive Biology (Fourth Edition). Academic Press, **2**: 23-50.

Sedighi, P., Helli, B., Sharhani, A. and Vatanpur, A. (2020). Effects of grape seed extract supplementation on fasting blood glucose, insulin resistance, and lipid profile in women with polycystic ovary syndrome. *Anatomical Science Journal* **17**(2):73–82.

Shen, M., Lin, F., Zhang, J., Tang, Y., Chen, W. K. and Liu, H. (2012). Involvement of the up-regulated FoxO1 expression in follicular granulosa cell apoptosis induced by oxidative stress. *Journal of Biological Chemistry*, **287**(31): 25727-25740.

Soleimani, M. and Masoumi, N. (2017). The effect of grape seed extract on semen oxidative stress markers in men with idiopathic infertility: a cross-sectional before-after study. *Nephro-Urol Mon*, ***9***: e13837.

Spicer, L. J. and Schütz, L. F. (2022). Effects of grape phenolics, myricetin and piceatannol, on bovine granulosa and theca cell proliferation and steroid production in vitro. *Food and Chemical Toxicology*, **167**: 113288.

Singh, J., Kaur, H., Kaur, R., Garg, R., Prasad, R., Assouguem, A., and Bahhou, J. (2023). A Review on the Nutritional Value and Health Benefits of Different Parts of Grape (Vitis vinifera L.). *Tropical Journal of Natural Product Research*, *7*(9).

Sun, Y. L., Tang, S. B., Shen, W., Yin, S. and Sun, Q. Y. (2019). Roles of resveratrol in improving the quality of postovulatory aging oocytes in vitro. *Cells*, **8**(10): 1132.

Tatsumi, T., Jwa, S. C., Kuwahara, A., Irahara, M., Kubota, T. and Saito, H. (2017). No increased risk of major congenital anomalies or adverse pregnancy or neonatal outcomes following letrozole use in assisted reproductive technology. *Human Reproduction*, **32**(1): 125-132.

Terra, X., Valls, J., Vitrac, X., Mérrillon, J. M., Arola, L., Ardèvol, A., Bladé, C., Fernández-Larrea, J., Pujadas, G., Salvadó, J. and Blay, M. (2007). Grape-seed procyanidins act as antiinflammatory agents in endotoxin-stimulated RAW 264.7 macrophages by inhibiting NFkB signaling pathway. *Journal of Agricultural and Food Chemistry*, **55**(11): 4357-4365.

Terral, J. F., Tabard, E., Bouby, L., Ivorra, S., Pastor, T., Figueiral, I., Picq, S., Chevance, J. B., Jung, C., Fabre, L. and Tardy, C., (2010). Evolution and history of grapevine (Vitis vinifera) under domestication: new morphometric perspectives to understand seed domestication syndrome and reveal origins of ancient European cultivars. *Annals of Botany*, **105**(3): 443-455.

Wang, J, Liu, D., Zhang, J., and Wang, Z. (2018). Resveratrol and Inflammation. *Journal of Agricultural and Food Chemistry*, **66**(2): 533-539.

Wang, Y., Nicholes, K. and Shih, I. M. (2020). The origin and pathogenesis of endometriosis. *Annual Review of Pathology: Mechanisms of Disease*, **15**(1): 71-95.

Wocławek-Potocka, I., Mannelli, C., Boruszewska, D., Kowalczyk-Zieba, I., Waśniewski, T. and Skarżyński, D.J., 2013. Diverse effects of phytoestrogens on the reproductive performance: cow as a model. *International Journal of Endocrinology*, **2013**(1): 650984.

Zhao, B. X., Sun, Y. B., Wang, S. Q., Duan, L., Huo, Q. L., Ren, F. and Li, G. F. (2013). Grape seed procyanidin reversal of p-glycoprotein associated multi-drug resistance via down-regulation of NF-κB and MAPK/ERK mediated YB-1 activity in A2780/T cells. *PloS one*, **8**(8): e71071.