Ginseng Extract Treatment Influences on Preimplantation Development in Vitro, and Pregnancy and Post-Partum Development in the Mouse

Danyka D. Belanger, The University of Western Ontario

Supervisor: Dr. Andrew Watson, The University of Western Ontario
A thesis submitted in partial fulfillment of the requirements for the Master of Science degree in Physiology
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GINSENG EXTRACT TREATMENT INFLUENCES ON PREIMPLANTATION DEVELOPMENT IN VITRO, AND PREGNANCY AND POST-PARTUM DEVELOPMENT IN THE MOUSE

(Spin Title: Ginseng Extract Treatment Influences on Preimplantation Development and Pregnancy)  
(Thesis Format: Monograph)

by

Danyka Danielle Belanger

Graduate Program in Physiology and Pharmacology

A thesis submitted in partial fulfillment Of the requirements for the degree of Master of Science

School of Graduate and Postdoctoral Studies The University of Western Ontario London, Ontario, Canada

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The thesis by

Danyka Danielle Belanger

entitled:

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is accepted in partial fulfillment of the requirements for the degree of Master of Science
ABSTRACT

Ginseng is one of the most commonly consumed herbal medicines in the world. Consumption is highest in females, thus my studies purpose was to define the concentration responsive effects of North American alcoholic and aqueous ginseng extracts on preimplantation development in vitro, and on pregnancy and post-partum development in the mouse. Two-cell embryos from female mice were cultured with five different concentrations of both extracts, ginsenosides Rb1, Rg1 and Re, a combinatorial ginsenoside solution and a polysaccharide fraction. Embryonic development and recovery from treatment was assessed. To investigate in vivo effects of ginseng extracts, females were gavaged with 50 mg/kg and 500 mg/kg of either extract (treatment), gavaged water (sham) or not gavaged (control) for two weeks prior to mating and throughout gestation. Gestation period, litter size and pup growth were evaluated. The results demonstrate that typical ginseng consumption levels do not adversely affect fertility or pregnancy in the mouse, however direct exposure to ginseng extract in vitro is detrimental to preimplantation development.

Key words: mouse, preimplantation development, ginseng, ginsenoside, North American ginseng, pregnancy, post-partum development, concentration-dependent developmental blockade, gavage, women
ACKNOWLEDGEMENTS

Deciding to do a masters degree has been one of the best decisions I’ve made, and this journey was made so memorable and enjoyable due to the people who have supported me through the past 2 years. Most importantly, I would like to thank my supervisor, Dr. Andrew Watson. Many graduate students would agree that the supervisor is imperative in setting the tone for your graduate studies experience, and Andy was constantly optimistic, encouraging and always put the student’s interest and needs before anything else. While he may not have been at the lab all the time, he never failed to make himself as available as possible. He helped and guided me along this journey, but also stepped aside and enabled me to take charge of my own learning experience, which has taught me more than he knows. I will always be grateful to him; his mentorship was invaluable to me. Secondly, I would like to thank the past and present members of the Watson lab, Michele, Christine, Paul, Sarah and Tamara. Working with you only enriched my experience at the VRL. I will always remember our friendships and times together, and wish you all luck and happiness in the future.

I would like to acknowledge my advisory committee members: Dr. Dean Betts, Dr. Edmund Lui, Dr. Morris Karmazyn and Dr. Valter Feyles. Thank you so much for your guidance and support through this process. Thank you to Dr. Andy Babwah for allowing me to use the phase contrast microscope and to Dr. Lui and Dr. Hua Pei for training me and letting me use the HPLC machine. I would also like to thank Trish and the vivarium staff for all of their help and hard work. They were always so accommodating, which made working with mice much easier.
When I decided to move my whole life from Ottawa to London, I admit, it was extremely scary. However the people I have met here and those who have accepted me into their lives have made these 2 years some of the best I’ve experienced. Thank you to Tamara, Ashley, Meaghan, Peter and Jeff for all of the great times we’ve shared together, you guys are my London family. Special thanks to Isabelle. Reconnecting with you after 6 years was the greatest surprise. Your friendship and support has meant the world to me. Congratulations, you are now stuck with me for life. A last thank you to my buddy, Leila.

My family is the most important thing in my life, and I would like to thank my parents, Jean-Marie and Carole for their endless support and confidence in my abilities, even when I didn’t necessarily believe in them myself. I now know, the sky is the limit. I would also like to acknowledge my two sisters and greatest allies, Jessica and Vanessa. While I may be the oldest sibling, the lessons I have learned from both of you are infinite and priceless.

Last but certainly not least; I would like to thank my best friend, partner in crime and boyfriend, Chris. This transition has been so much more positive and enjoyable because of you. Your continued support and encouragement through these past 7 years have been a great factor in my success and I would like to thank you for always being there for me.

Finally, I would like to dedicate my thesis to my uncle Jacques, who will forever be in my thoughts, and whose guidance helped shape the person I am today.
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LIST OF ABBREVIATIONS

α alpha
ALC alcoholic
AQ aqueous
β beta
BP blood pressure
°C degrees Celsius
Ca$^{2+}$ calcium ion
CD1 cluster of differentiation
cGMP cyclic guanosine monophosphate
CO$_2$ carbon dioxide
DMBA 7,12-dimethylbenz(a)anthracene
EPC ectoplacental Cone
ExE extra-embryonic ectoderm
FDA food and drug administration
g gram
GABA gamma-aminobutyric acid
hCG human chorionic gonadotropin
HIV human immunodeficiency virus
h hour
HRA human ovarian cancer
ICM inner cell mass
IGF insulin-like growth factor
<table>
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<tr>
<td>IGF-II</td>
<td>insulin-like growth factor II</td>
</tr>
<tr>
<td>IGFBP-I</td>
<td>insulin-Like growth factor binding protein-I</td>
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<tr>
<td>IL-1β</td>
<td>interleukin-1β</td>
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<tr>
<td>kg</td>
<td>kilogram</td>
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<tr>
<td>KSOMaa</td>
<td>potassium simplex optimized medium with amino acids</td>
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<td>min</td>
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<td>mL</td>
<td>milliliter</td>
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<td>MMP-2</td>
<td>matrix metalloproteinase 2</td>
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<td>MMP-3</td>
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<td>MRP</td>
<td>maillard reaction product</td>
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<td>N₂</td>
<td>nitrogen</td>
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<td>NAG</td>
<td>north american ginseng</td>
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<td>NO</td>
<td>nitric oxide</td>
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<td>O₂</td>
<td>oxygen</td>
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<td>protopanaxadiol</td>
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<td>prostaglandin</td>
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<td>PGF2α</td>
<td>prostaglandin F2α</td>
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<td>PMSG</td>
<td>pregnant mare serum gonadotropin</td>
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<td>PS</td>
<td>polysaccharide</td>
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<td>PSF</td>
<td>polysaccharide fraction</td>
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<td>PT</td>
<td>protopanaxatriol</td>
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<tr>
<td>RM ANOVA</td>
<td>repeated measure analysis of variance</td>
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<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>TE</td>
<td>trophectoderm</td>
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TJ  tight junction
µg  microgram
µL  microliter
US  united states
UWO  The University of Western Ontario
**Introduction**

1.1 **Rationale - Overview**

Herbal medicinal products are a growing market due to their increased integration into peoples every day lives. In the United States (US), their consumption has increased by 380% in the general population between 1990 and 1997 (Eisenberg et al. 1998). Additionally, in 1994 the total US sale of herbal products was $4 billion (Brevoort 1998). Among these popular herbal remedies is ginseng, whose consumption and inclusion as part of a healthy diet is on a dramatic rise due to its many purported health benefits, making it one of the 10 most commonly used herbal medicines in the United States (Bent and Ko 2004). Ginseng is an herb in the Araliaceae family and is a species in the genus Panax, which means, “all healing” and “longevity”, and while its medicinal properties have been appreciated in Asia for over 2000 years, it is now becoming increasingly important across the world, specifically in North America (Chu et al. 2009).

Research focused on investigating the therapeutic effects of ginseng has considerably increased due to its ever-growing popularity as an herbal remedy. Research is required to ensure that human consumption of ginseng is safe and to define the mechanisms underlying its beneficial effects. Studies have demonstrated that ginseng extracts are composed of many compounds with unique and beneficial influences on various body systems, including the cardiovascular system, nervous system and the reproductive system (Attele et al. 1999). However, as herbal remedies are usually deemed harmless because they are “natural”, this does not in any way ensure that normal levels of consumption
of these products are safe (De Smet et al. 2004). In a US survey conducted in 2001, 9.1% of 242 pregnant women questioned declared use of herbal medicinal products during their current pregnancy, and 7.5% were using these supplements at least weekly. The most commonly used products were garlic, aloe, chamomile, peppermint, ginger, Echinacea, pumpkin seed and ginseng (Gibson et al. 2001). In Asia, up to 10% of women have reported use of ginseng through out their pregnancy (Chin 1991). With more women of reproductive age taking herbal supplements, it is important to investigate ginseng’s effect and safety on preimplantation development and pregnancy.

To date, research investigating ginseng’s impact on reproductive function has demonstrated a positive effect on sperm motility (Zhang et al. 2006), but also lower morphological scores for \textit{in vitro} treated rat and mouse embryos than for those cultured in a normal environment (Chan et al. 2003, 2004; Liu et al., 2005a, 2005b, 2006). This later outcome is quite controversial since Lee et al. 2008 found that treatment of early rat embryos with individual ginsenosides caused either no significant change to the morphological scores within the organogenic parameters used, and in some cases actually improved them. Ginseng may also protect mouse embryos from oxidative stress due to ethanol-induced teratogenesis during embryonic organogenesis via activation of embryonic antioxidant enzyme defense systems (Lee et al. 2009). In addition, very recent \textit{in vivo} studies have shown that when administering 20, 200 and 2000 mg/kg/day (2000 mg/kg/day being \approx 200 times the recommended clinical dose) of Korean Red Ginseng Extract to females for two weeks before mating and through out gestation, that maternal weight, feeding, embryonic implantation and fetal growth
were not significantly affected (Shin et al. 2010). While research in this field is advancing quickly, it is important to establish whether consumption of all ginseng extracts are safe during pregnancy and what effects, if any, direct exposure to these extracts may have on preimplantation development. Furthermore, due to so much variability between the compounds found in different ginseng products (Liberti and Der Marderosian 1978), it is important to study the effects of different ginseng species and extracts on reproduction, which is why the aqueous and alcoholic extracts produced from North American ginseng (NAG) are used in this research.

Addressing these concerns were the primary purpose of my study. I investigated the effects of aqueous (AQ) and alcoholic (ALC) NAG extracts and individual ginsenosides and polysaccharides on preimplantation development in vitro. I also investigated the influences of both ginseng extracts on fertility, pregnancy and post-partum development in the mouse. I have applied my studies to the mouse since it is a well characterized research model for understanding mammalian development. Furthermore, it is critical to conduct animal model studies prior to trials applied directly to the human.

1.2 Embryonic Development: From Fertilization to Childbirth

1.2.1 Preimplantation Development

Preimplantation development encompasses the developmental period from fertilization to embryo implantation into the uterine wall (Watson and Barcroft 2001). Fertilization occurs in the swollen ampulla of the oviduct, also referred to as the fallopian tube in the human, and includes the fusion of a sperm with a
mature oocyte released from the ovary. The embryo travels down the oviduct towards the uterus. The first cleavage division occurs 16-20 hours following fertilization and is characterized by an asynchronous doubling of the number of cells, producing a short period of time where cell number may not be even. Cleavage produces an embryo with two cells of approximately equal size called blastomeres (Fujimori 2010). The first major developmental transition that occurs during these early stages of development is the zygotic gene activation, where the developmental program of the embryo is no longer exclusively directed by maternal proteins and transcripts, but by newly expressed genes (Schultz 2002).

Individual blastomeres are easily distinguished until half way through the eight-cell stage in the mouse when compaction, the first morphological event, transpires. Compaction is characterized by an increase in cell-cell adhesion due to E-Cadherin and tight junction (TJ) formation, (Shirayoshi et al. 1983; Watson and Barcroft 2001) causing the membranes of individual blastomeres to be indiscernible. Compaction is also linked to the onset of cellular polarization of the outer cells of the embryo, (Watson and Barcroft 2001) which form the trophectoderm (TE). Additionally, the inner cells give rise to the Inner Cell Mass (ICM) (Johnson and McConnell 2004). At the 32-cell stage, multiple small fluid filled cavities begin to form in the embryo due to active transport of ions and subsequent fluid movement down the ionic concentration gradient across the trophectoderm epithelium, a phenomenon known as cavitation. The individual cavities fuse together and create the blastocyst cavity, which separates the two cells types. The ICM cells are pluripotent and become the embryo proper, whereas the TE gives rise to the placenta. These final steps lead to the formation
of the blastocyst, which precedes embryo implantation to the uterine wall and is the last step of preimplantation development (Enders and Shlafke 1968; Lin et al. 2001; Watson and Barcroft 2001; Fujimori 2010).

1.2.2 Implantation

Implantation is the attachment of the embryo to the maternal endometrial epithelium and its ensuing invasion into the stroma (Hemberger 2012). The TE, previously referred to as the outer cell layer of the embryo, is made up of trophoblast cells, and its initial role is to mediate implantation of the blastocyst into the uterine wall (Enders and Shlafke 1968). Before invasion by trophoblast cells begin however, maternal endometrial stromal cells transform into decidual cells and form a dense cellular matrix called the decidua. This physical barrier slows embryonic migration toward the mother’s uterine spiral arteries (Kearns and Lala 1983) and promotes trophoblast attachment rather than invasion (Kliman 2000). The decidualized stromal cell is also a secretory cell and expresses new proteins such as prolactin and insulin-like growth factor binding protein-1 (IGFBP-1) (Tarantino et al. 1992). After decidualization, the trophoblast cell differentiation pathway is altered, and villous trophoblasts are transformed into anchoring trophoblasts (Kliman 1994; Kliman 1999) in part due to contact with the decidua. Trophoblasts then migrate to the uterus and direct their movement toward the maternal spiral arteries where their cellular extensions form an anastomosing network around the implantation site. This allows permeation of the attachment site with maternal blood via low resistance channels. Finally, trophoblasts limit their invasion to the upper third of the uterus and are eliminated.

While implantation is extremely complex and not all compounds involved have been discovered, the function of many proteins and cytokines is known. Interleukin-1, specifically interleukin-1β (IL-1β), is proposed as one of the factors mediating cross-communication between the maternal endometrium and the implanting blastocyst (Simon et al. 1994; Simon et al. 1997). IL-1β also induces expression of IGFBP-I, which allows dissociation of filamentous actin during decidualization (Kim et al. 1999) and MMP-3, which degrades several components of the extracellular matrix and basement membrane thus allowing decidualization to ensue (Hemberger 2012). Lastly, insulin-like growth factor (IGF) signaling systems allow cell-cell communication between fetal trophoblasts and maternal decidual cells by interactions between IGF-II and IGFBP-I, (Hamilton et al. 1998) which may act synergistically to enhance trophoblast migration. In conclusion, the fate of implantation is likely due to the balance between the invasive promoting protease generated by trophoblasts and the inhibitors of invasion created by the decidua (Feinberg et al. 1989; Graham and Lala 1991; Strickland and Richards 1992; Kliman 1994).

1.2.3 Embryogenesis

Embryogenesis is the progression of events adhered to by the embryo in order to form and develop into a healthy baby. Initially, the TE gives rise to the chorion, the precursor of the placenta. The ICM contributes to the embryo proper and gives rise to the yolk sac, allantois and amnion. Following implantation, the
embryoblast forms a bilaminar embryonic disc called the embryonic epiblast, which provides most of the cells contributing to the embryo proper (Hall 1999, 2000). Afterward, gastrulation occurs, where two germ layers are generated from the single layered blastula, the inner ectoderm and outer endoderm. The mesoderm, the third and middle germ layer, forms secondly (Hall 1999, 2000). Each germ layer differentiates into different organs and tissues in the body. The ectoderm becomes the epidermis of the skin and the neural tissue. It also differentiates into numerous crucial reproductive organs such as the mammary glands, the hypothalamus, both pituitary lobes, the caudal vagina, the vestibule, the penis and the clitoris (Gilbert 2000). The endoderm forms the lining of the gastrointestinal and respiratory tract as well as their derivatives. Finally, the mesoderm differentiates into the urogenital, circulatory and supportive muscular system as well as the female and male gonads, the uterus, the cervix, the cranial vagina, the epididymis and the accessory glands (Senger 2003; McGeady et al. 2006). Additionally, a fourth germ layer arises during primary neurulation called the neural crest (Hall 1999, 2000). Once all germ layers have been established, organogenesis begins. Organogenesis is the process through which individual germ layers develop into the internal organs of the body, and is initiated by secondary neurulation and the formation of the neural tube. Also, blastema cells (cells capable of growing and regenerating into bodily tissues and organs) differentiate into the hindgut, blood vessels, somites, trunk neural crest cells, and the notochord (Hall 1998; O’Rahilly and Müller 1999). At this point, organ development continues and includes formation of the central nervous system, the epidermis, cutaneous structures, the skeletal and muscular system, the heart,
blood vessels and cells, the respiratory and gastrointestinal tract, sex organs and all other systems in the body (Gilbert 2000).

1.2.4 **Placentation**

Placentation is the formation and arrangement of the placenta. In the mammal, as formerly described, the placenta is derived from the trophoblast cells of the TE (Johnson and McConnell 2004). Immediately after implantation, TE cells that are not in direct contact with the ICM differentiate into highly polyploid trophoblast giant cells that line the entire implantation site (Sutherland 2003; Hemberger 2012). These cells, along with a juxtaposed layer of endodermal cells, form the parietal yolk sac, (Chazaud et al. 2006) which mediates nutrient, gas, and waste exchange between the mother and embryo before the mature placenta takes over on embryonic day 9.5-10.5 (Hemberger 2012). Furthermore, the parietal trophoblast giant cells protect the embryo through a microbial defense system and shield the embryo from immune rejection by the mother (Amarante-Paffaro et al. 2004). Contrastingly, the TE cells that are in contact with the ICM continue to proliferate, give rise to extra-embryonic ectoderm (ExE) cells, and migrate into the ectoplacental cone (EPC) (Hemberger 2012). At this stage, the peripheral cells cease to proliferate. They become larger and less congested and differentiate into a secondary population of trophoblast giant cells (Parast et al. 2001; Sutherland 2003). This proliferative zone is necessary to provide a sufficient number of cells to form the future placenta.

In the mouse, the placenta forms around mid-gestation into three layers: the maternal decidua, the junctional zone and the labyrinth (Hemberger 2012).
The functions most attributed to the placenta are nutrient and gas exchange, which are accredited to the labyrinth in the mouse, and the chorionic villi in the human (Rossant and Cross 2001). This layer is formed by fusion of the allantois and the chorion, and branches out at defined initiation sites to establish an intricate fetal vascular network. This network ensures that the maternal and fetal blood circulation come into very close contact, but never intermingle, ensuring continued fetal growth (Hemberger 2012).

1.2.5 Parturition

Parturition is the step-by-step process of giving birth, and is the last stage in fetal development. There are five independent physiological actions that constitute parturition: fetal membrane rupture, cervical dilatation, myometrial contractility, placental separation and finally uterine involution (Olson et al. 1995). These events result in the birth of the baby and the restoration of a regular cyclical uterine physiology. The process by which parturition is initiated has been extensively investigated. Pregnancy is characterized by long-duration and low-amplitude contractions in the myometrium, whereas contractions become shorter, high amplitude and synchronous throughout the entire muscle during active labor (Taylor et al. 1983). Uterine quiescence is a state maintained throughout pregnancy, and is controlled by high concentrations of progesterone. When progesterone quantities are elevated, the myometrium does not contract when stimulated by oxytocin or PGF2α (Challis and Olsen 1988). However, the dormancy state is lost during childbirth, and labor initiation is suggestively controlled by a reduction in the concentration of circulating progesterone (Csapo
Uterine activation and stimulation are considered two separate but parallel physiological events (Lye and Challis 1989). To begin, uterine activation is considered to be the result of a relative surge of estrogen at term in the face of a high progesterone environment (Albrecht and Pepe 1990; Olsen et al. 1995). Activation incorporates many modifications in myometrial cells, such as resting membrane potential changes, augmented levels of myosin and actin, elevated gap junction, oxytocin and prostaglandin (PG) receptor expression and finally an increase of post-receptor coupling mechanisms (Olsen et al. 1995). Interestingly, PGs have been shown to have a role in uterine activation by stimulating the formation of gap junctions in rat myometrium (Garfield et al. 1980). Secondly, uterine contractile stimulation is linked to increasing myometrial responsiveness to oxytocin in late gestation (Nathanielsz and Honnebier 1992; Hirst et al. 1993). Hirst et al. 1991 discovered that the intensity and duration of contractions amplified in parallel with growing levels of oxytocin in rhesus monkey plasma. While oxytocin is not responsible for the onset and timing of labor, it contributes to the expulsive phase of parturition. Moreover, PGs are also essential in maintenance of labor, and like oxytocin, their levels rise after labour has started and through out its progression. Lastly, hormones initiating, activating and stimulating parturition may be coordinated in part by an intrauterine communication system composed of steroids, cytokines and PGs (Keirse 1990; Olsen et al. 1995). While research is quickly advancing, the process of parturition is complex and inadequately understood, requiring additional studies before a complete understanding of the mechanisms controlling labor are effectively known.
1.3 Herbal Medicines and Pregnancy

Intriguingly, most survey data indicate that women are the predominant users of herbal medicines (MacLennan et al. 1996; Eisenberg et al. 1998; Ernst and White 2000), and as previously indicated, 9.1% of pregnant women take herbal supplements throughout their pregnancy (Chin 2001). The most alarming fact surrounding these statistics however, is the lack of accessible resources and information regarding the safety of consuming herbal supplements during pregnancy. While information may be preliminary, herbs have been associated with possible health risks throughout childbearing (Ernst 2002).

The definition of a word helps people understand its significance and importance. Due to the fact that herbal remedies are characterized as “supplements” and are deemed “natural”, consumers often believe that they are inherently safe and do not have similar properties to drugs that can have biological and clinical effects when ingested. This of course is a false assumption. The general population needs to be careful when contemplating herbal remedies (Stein 2002). In many countries, including the United States, natural products are not rigorously regulated, and people may not necessarily know what they are consuming (De Smet et al. 2004). It is clear that these products can have clinical effects on the body, however because they are not classified as drugs, they do not require approval or rigorous testing by the FDA before they are put on the market (Stein 2002). Furthermore, the only way the FDA is empowered to remove herbal remedies from the consumer’s reach is if they are proven unsafe rather than initially being proven safe by the manufacturer (Goldman 2001).
Undeniable characteristics of herbal remedies mark them and their potential therapeutic effects as unpredictable, as follows:

Phytochemical studies have indicated that the concentration of the principal compounds present in several herbal supplements on the market, such as St. John's wart, ginko, Echinacea, soy and ginseng, vary dramatically from product to product (De Smet et al. 2004). For example, hypericin is a bioactive compound found in St. John’s Wart. On the label of different products found at a local health food store, it was stated that levels of 0.3% hypericin differed for many products and ranged between 180mg and 530mg. Other labels listed hypericin but did not include the concentration or amount of active ingredient in the product or simply stated that parts of the plant were used but not the specific compounds found in them. Finally, while some labels did indicate an awareness of certain studies performed on hyperforin, others indicated that no barriers exist to selling herbal remedies of uncertain potency and safety (Goldman 2001).

Unfortunately, there are many limitations to our understanding of all the active compounds found in herbal products, and setting a standard for each of them individually would be extremely challenging if not near impossible.

Chemical analysis is no longer valuable when the ingredients responsible for a plant’s activity are unknown and have not been identified. On another note, even if these compounds are identified, it is difficult to assess if the crude herb would be preferable to ingesting the purified active compound (Goldman 2001). While a method of standardization for compounds found in herbal products would be indispensable for their quality and safety, any guidelines put into place would be futile without complete adherence of future regulations by the manufacturers.
as well as supervision at the retail level. In a Canadian study conducted by Mills et al. 2003, simulated customers visited 33 health food stores one month after Health Canada published a warning about the hepatotoxic potential of kava. It was found that 67% of these stores were still selling kava. When the Canadian ban on kava was made official and 30 stores were revisited two months later, it was shown that 57% were still selling the dangerous supplement.

Many herbal supplements produce undesirable effects in the body such as headaches, vaginal spotting, and gastrointestinal discomfort (De Smet et al. 2004). As previously stated, these products are often believed by the public to be safe due to their natural and organic origins (De Smet et al. 2004). Largely due to this misconception, half of the herbs taken by patients are not reported to their physicians, thus a correlation between consumption of natural health supplements and some of the adverse effects experienced are not commonly understood (Foster et al. 2000). Furthermore, although no reliable figures about underreporting of herbal adverse outcomes are available, it has been suggested that less than 1% of undesirable incidences associated with herbal remedies are reported to the FDA in the United States (Marcus and Grollman 2002). This implies that most of the information regarding the harmful effects produced by these products is not available to the general population, which prevents the public from making educated choices when deciding to incorporate herbal supplements into their diets. An additional serious factor when considering the consumption of herbal supplements is the likely presence of contaminants and adulterants such as high levels of pesticides, fumigation agents, pathogens, microbial toxins or toxic botanicals (De Smet et al. 2004). Particularly concerning
is the presence of toxic levels of heavy metals such as mercury, lead and arsenic in several Asian medicines purchased in Asian health food stores in the USA, even when taking the suggested dose found on the package (Garvey et al. 2001). These adulterants are rarely listed on labels, which make them especially dangerous. In a California study of Asian medicines purchased from herbal stores, 32% of a 260 product sample contained undeclared pharmaceuticals or heavy metals, and 23 had more than one adulterant (Ko 1998). Among the contaminants found in some Asian herbal remedies are also synthetic pharmaceuticals such as antihistamines, aromatics, steroids and alkaloids (Joubert and Mathibe 1989; Gorey et al. 1992; Chen et al. 1993).

Unknown additives in health products are a cause for concern, and the industry clearly states that not all compounds and chemicals present in supplements are known or advertised. This indicates that the public must approach the ingestion of herbal products with great caution, not only for personal knowledge and safety but also due to possible dangerous consequences, such as drug interactions. To illustrate, in a group of normal volunteers being administered St. John’s wort for 10 days, it was revealed that the herbal product reduced absorption of the drug digoxin by an average of 25% (Johne et al. 1999). Furthermore, case reports also indicated a significant increase in the metabolism of other drugs when ingesting St. John’s wort, including cyclosporine (Ruschitzka et al. 2000), warfarin (Yue et al. 2000), and oral contraceptives (Ernst 1999). While many people have readily welcomed the opportunity to gain access to natural remedies claiming to deliver solutions to some of their health concerns, it may not be so simple due to the lack of reliable
information provided about these products (Goldman 2001).

1.4 Properties of Ginseng and Ginsenosides

Ginseng is an herbal supplement with a complex chemical composition. Established compounds are extensive and include polysaccharides (PS), peptides, polyacetylenic acids, saponins, fatty acids and ginsenosides (Lee 1992). It is suggested that these different composites differ depending on the species, root age, cultivation time and the method of production (Liberti and Der Marderosian 1978). Due to production method variation, it is clear that steamed ginseng products have greater pharmacological potency than non-steamed ginseng as well as differing bioactive components (Yun 2003). For instance, using heat (Kim et al. 2000) or sonication (Fuzzati et al. 2000) increases ginsenoside recovery and biological activity. Specific ginsenosides and their concentrations will also differ when looking at distinctive ginseng products. For example, 25 ginseng products purchased from health food stores were tested for product-to-product variability in ginsenoside concentration. It was found that ginsenoside quantities varied 15-fold (0.288-4.255% by weight) in the powders and capsules per 100g of product, and 36-fold (0.361-12.993 g/L) in the liquid extracts for 100mL of product (Harkey et al. 2001). Finally, the unique chemical structure of each individual ginsenoside along with the different combination of compounds found in each specific species are what produce different pharmacological and physiological effects in the body (Sievenpiper et al. 2003) such as antioxidant, anti-inflammatory and anti-carcinogenesis benefits (Wargovich 2001; Kang et al. 2007; Lee et al. 2009;). The ginsenosides are the
principal bioactive constituents of interest for understanding ginseng medicinal effects. Approximately 40 ginsenoside compounds have been identified from the ginseng root and their separation and analysis methods have been extensively reviewed (Fuzzati 2004).

The most common types of ginseng are American ginseng (Panax quinquefolium L.) and Asian ginseng (Panax ginseng C. A. Meyer) (Lü et al. 2009). North American ginseng (NAG) has a similar ginsenoside profile to Asian ginseng. Some marked differences however include a much higher concentration of ginsenosides Rb1 and Re in the root of NAG, and little to no Rf (Hu and Kitts 2001). In addition, Dr. Edmund Lui at the University of Western Ontario has determined that the ALC extract derived from NAG possesses twice the total concentration of ginsenosides as the AQ extract, specifically 282.5 mg/g versus 138.7 mg/g. It was also found that the ALC extract has more Rb1 and Re, but a lower concentration of Rg1 than the AQ extract. In addition, the major difference between both extracts is that the AQ extract is composed of 15% polysaccharides, whereas the ALC extract has none. Ginsenosides belong to a family of steroids called triterpene saponins and have a four-ring, steroid like structure with sugar moieties attached. Sugar moieties include but are not restricted to glucose, maltose, fructose and saccharose and are attached to C-3, C-6 and C-20 of the chemical structure (Attele et al. 1999). Each one also has at least two or three hydroxyl groups and depending on their aglycone are either derivatives of the (20S)-protopanaxadiols (PD), (20S)-protopanaxatriols (PT), (Tawab et al. 2010) acotillol-type or oleanolic acid groups (Fuzzati 2004).
Figure 1.1  Chemical Structures of Main Ginsenosides and their Key Degradation Products.

Chemical structure and m/z values of main ginsenosides in the protopanaxadiol and protopanaxatriol groups, as well as their key degradation products. Also, major fragment ions detected in the initial fragmentation step (MS²).
**Ginsenoside Structural Diversity**

![Ginsenoside Structural Diagram](image)

<table>
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<tr>
<th>Compound</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>m/z</th>
<th>m/z of major fragments</th>
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<td></td>
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<td>-H</td>
<td>-O-Glc⁶,¹Glc</td>
<td>1131</td>
<td>789, 365</td>
</tr>
<tr>
<td>G-Rb₂</td>
<td>-O-Glc³,¹Glc</td>
<td>-H</td>
<td>-O-Glc⁶,¹Arap</td>
<td>1101</td>
<td>789, 335</td>
</tr>
<tr>
<td>G-Rc</td>
<td>-O-Glc³,¹Glc</td>
<td>-H</td>
<td>-O-Glc⁵,¹Araf</td>
<td>1101</td>
<td>789, 335</td>
</tr>
<tr>
<td>G-Rd</td>
<td>-O-Glc³,¹Glc</td>
<td>-H</td>
<td>-O-Glc</td>
<td>969</td>
<td>789</td>
</tr>
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<td>-H</td>
<td>-OH</td>
<td>461</td>
<td>443, 425, 407</td>
</tr>
<tr>
<td>Compound-K (C-K)*</td>
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<td>-H</td>
<td>-O-Glc</td>
<td>645</td>
<td>203</td>
</tr>
<tr>
<td><strong>Protopanaxatriol-type</strong></td>
<td></td>
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<tr>
<td>Ginsenoside G-Re</td>
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<td>969</td>
<td>789</td>
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<td>-O-Glc</td>
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<td>643</td>
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<tr>
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<td>-OH</td>
<td>807</td>
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<tr>
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<td>-OH</td>
<td>823</td>
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<tr>
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<tr>
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<td>-OH</td>
<td>-O-Glc</td>
<td>661</td>
<td>203</td>
</tr>
<tr>
<td>20(S)-protopanaxatriol</td>
<td>-OH</td>
<td>-OH</td>
<td>-OH</td>
<td>477</td>
<td>459, 441, 423</td>
</tr>
</tbody>
</table>

1.5 Pharmacokinetics of Ginseng

The precise plasma level of ginseng and intact ginsenosides in the human body after daily ginseng consumption is undetermined, as well as whether absorption of intact ginsenosides by the human gastrointestinal tract is possible (Tawab et al. 2003). It is now clear that PDs such as Rb1 are converted to compound-K by enzymes from intestinal bacteria (B-D-glucosidase) through step wise cleavage of the sugar moieties, (Hasegawa et al. 1996) and PTs such as Re and Rg1 are hydrolyzed into ginsenoside Rh1 and Rf1 under mild acidic conditions (Han et al. 1982). This results in a small percentage of intact ginsenosides being absorbed by the gastrointestinal tract after oral administration in humans and animals. While many have attempted to discover these ginsenoside levels, results often vary. The individual amount of each identified ginsenoside and their metabolites in plasma are not known, however, it was found that the maximum concentrations of ginsenosides R1, Rg1, Rd, Re and Rb1 in rat plasma after an oral administration of 10 and 300mg/kg of ginseng powder were between 1.51 to 6.42 µg/ml on average when samples were taken regularly over four days (Li et al. 2007a). Additionally, their absolute bioavailability was 9.29%, 6.06%, 2.36%, 7.06% and 1.18% respectively (Li et al. 2007b). Levels of Rb1 and Rg1 in the plasma have also been determined for pregnant female mice on gestational day 18 by Shin et al. 2010. Standard solutions were made and 10, 100, 500, 1,000, and 2,000 µg of ginsenoside Rb1 and Rg1 were dissolved in methanol, filtered and 20µl of each solution was analyzed by HPLC. It was found that their concentrations in Korean Red Ginseng Extract are 0.61±0.12% and 0.9±0.17% respectively. However in this case, while
Rb1, Rg1 and Re were detected after spiking in normal serum, no trace of these ginsenosides were found in plasma following treatment with the highest dose of 2000mg/kg/day after 30 min and 2h. It was suggested that these ginsenosides do not reach a detectable blood level by oral treatment due to low absorption in the gastrointestinal tract.

In the human, Cui et al. 1997 demonstrated that ginsenosides are present in urine after oral consumption. They demonstrated that 1.2% of a three mg dose of orally ingested protopanaxatriol ginsenosides and 0.2% of a seven mg dose of orally ingested protopanaxadiols were recovered in the urine over five days. Yet, neither the ginsenoside nor its metabolite could be identified, simply the aglycone. Ginsenosides Rg1, Rd, Re, Rb2 and Rc are more easily detected in urine than in plasma, which may be attributed to the lower limit of detection present in urine and indicates that their concentrations in plasma must be extremely low. It has been documented that even though low levels of intact ginsenosides are found in mouse plasma, that only ginsenoside Rb1 was detectable in human blood, and by only one subject (Tawab et al. 2003). This is due to the fact that only hydrolyzed and metabolized compounds are able to reach the systemic circulation. The only degradation products to reach circulation were Rh1 and Rf1 derived from PTs and compound-K originated from PDs digested in the intestine (Tawab et al. 2003). With the exception of Rb1, it was established that high levels of intact ginsenosides are not able to reach the plasma because they are first metabolized. Additionally, while it has been discovered that low ginsenoside accumulation and bioavailability are due to
decomposition in the stomach, metabolism in the intestine and elimination in the liver, it is believed that low membrane permeability is the dominant factor (Han and Fang 2006). These results indicate that ginsenoside pharmacokinetics are incredibly variable from one study to another and from one species to another. Thus pharmacokinetics of ginseng extracts and placental transfer of ginsenosides in humans must be investigated further.

1.6 Beneficial Effects of Ginseng and Ginsenosides

The scientific literature supports that ginsenosides are responsible for most of ginseng’s medicinal activities; however non-ginsenoside compounds also exert certain pharmacological effects, indicating that the whole root can offer therapeutic advantages (Lü et al. 2009). Ginseng is one of the more frequently purchased herbs in the US and has been used as an herbal supplement for hundreds of years by people wanting to boost energy levels, reduce stress and possibly increase lifespan by maintaining a healthy lifestyle (Angelova et al. 2008). People with certain illnesses also use ginseng. For instance, patients with cardiovascular disease and HIV use this herbal remedy in conjunction with their treatments. A Canadian survey conducted in 2003 found that 6% of patients with cardiac illnesses reported use of ginseng (Wood et al. 2003). Interestingly, ginseng is also used by 34% of HIV positive patients on antiretroviral therapy making it the 10th most used complementary and alternative medicine (Standish et al. 2001). Due to the overall activity and complexity of the herb, its use to target benefit to a wide number of the various systems of the body is not surprising.
1.6.1 **Reduction of Blood Glucose and Improvement of Diabetes**

Diabetes is a major global health problem affecting about 3% of the world population (Park et al. 2005). Of this 3% worldwide, over 90% of diabetic patients have type two diabetes, which is mostly related to aging, diet and lifestyle. It can cause serious health complications and can reduce life expectancy by 8-10 years (Astrup and Finer 2000). Fortunately, it has been observed that the root of Panax ginseng can be used to improve glucose homeostasis and insulin sensitivity (Sonnenborn and Proppert 1990). Genetically obese diabetic mice treated with a single 90mg/kg dose of ginseng root extract by intraperitoneal injection had significantly lower glucose blood levels (Kimura et al. 1999). Furthermore, when type two diabetic patients and non-diabetic patients were administered three g of American ginseng root 40 minutes before a test meal, it was demonstrated that blood glucose levels were lowered in both patient groups (Vuksan et al. 2000). Ginseng therapy for type two diabetic patients has been shown to significantly lower blood sugar levels, but it additionally reduces fasting blood glucose and body weight, elevates mood and improves psychophysical performance (Park et al., 2005). These observations signify that ginseng may not only be beneficial for type two diabetic patients, but also more surprisingly for the non-diabetic population as a preventative measure against developing diabetes in the future.

1.6.2 **Antihypertensive Effects**

High blood pressure (BP) is associated with many life threatening diseases and is correlated with an augmented risk of stroke, coronary heart disease and end-organ illnesses such as renal failure (Park et al. 2005).
Intracellular Ca\textsuperscript{2+} availability regulates blood vessel smooth muscle tone and is strongly influenced by cellular membrane and sarcoplasmic reticulum interaction in the muscle. In order to establish the effectiveness of a particular drug on high BP, its influence on the smooth muscle can be analyzed (Lee 1980). It was discovered that protopanaxadiols (PD) and protopanaxatriols (PT) inhibit Ca\textsuperscript{2+} binding to the cellular membrane, and that PT ginsenosides were approximately 180% more effective (Lee 1980). Ginseng consumption is thus linked with normalizing the BP of hypertensive patients (Yammamoto 1992). Additionally, ginsenosides also lower BP in a dose dependent manner in rats administered doses of 10-100mg/kg by mediating the release of endothelium-derived nitric oxide (NO). NO increases the accumulation of cGMP and in turn relaxes blood vessels (Kim et al. 1994).

Ginseng is also a potent antiplatelet agent (Teng et al. 1989). Endothelial cell damage is the first step in the genesis of thrombosis and arteriosclerosis, common precursors of cardiovascular disease. Platelet hyperfunction and aggregation is associated with an overproduction of Thromboxane A2, which occurs in patients with cardiovascular thrombotic disease (Park et al. 2005). Panaxynol is the principal compound in ginseng that exhibits antiplatelet effects by preventing thromboxane production (Teng et al. 1989). It was also revealed that panaxynol inhibits aggregation, and thromboxane release and formation in rabbit platelets, whereas ginsenosides Ro, Rg1 and Rg1 blocked release only (Kuo et al. 1990). These results certainly suggest that ginseng may have a therapeutic role in offsetting or even treating various cardiovascular diseases.
1.6.3 **Antioxidant Effects**

Ginseng has demonstrated protective effects in the body attributed to its antioxidant activities. It has been revealed that at concentrations between 50-100ug/ml, ginseng can act as a free radical scavenger or hydrogen donor. Phenolic contents and MRP levels (Kang et al. 2007) or ginsenosides have been suggested as the active compounds responsible for this observed phenomenon (Hu and Kitts 2001). Administration of American ginseng was shown to improve antioxidant enzyme activity in rats for enzymes such as superoxide dismutase and glutathione peroxidase (Fu and Ji 2003). American ginseng has also been proven to have a protective effect against free radical damage on myocardial cells induced by xanthine by countering the action of free radicals (Zhong and Jiang 1997). Moreover, ginsenoside Re has been revealed to exert antioxidant properties in cardiomyocytes (Xie et al. 2006) and neuroprotective influences on cellular damages stimulated by amyloid and serum free medium (Ji et al. 2006). Additionally, it has been confirmed that ginsenoside Rd can improve astrocyte differentiation from neural stem cells (Shi et al. 2005). By increasing antioxidant
enzyme levels and acting as a free-radical scavenger, ginseng and ginsenosides display protective antioxidant effects on several systems in the body.

1.6.4 The Central Nervous System

Ginseng can wield both stimulatory and inhibitory outcomes on the central nervous system (Saito et al. 1977), and may have a regulatory role on neurotransmission. Ginsenosides Rb1 (Benishin et al. 1992), Rg1 (Yamaguchi et al. 1995) and Re (Yamaguchi et al. 1996) have preventative effects against scopolamine-induced memory deficits. Central cholinergic systems are involved in mediating learning and memory processes (Perry 1986). It was disclosed that Rb1 could partially reverse scopolamine-induced amnesia by increasing cholinergic activity (Takemoto et al. 1984; Salim et al. 1997) via augmented uptake of choline in cholinergic nerve endings (Benishin 1992) and aiding in the release of acetylcholine from hippocampal slices (Benishin 1992). These studies signify that ginsenosides may improve memory, facilitate learning and effectively enhance nerve growth (Takemoto et al. 1984; Salim et al. 1997). On another note, ginsenosides can protect neurons from ischemic damage. It was revealed that Rb1 rescued hippocampal neurons from fatal ischemic damage (Lim et al. 1997) and was able to delay neuronal death from transient forebrain ischemia (Wen et al. 1996). Finally, ginseng extract is also associated in nerve transmission modulation by decreasing neurotransmitter availability. It was demonstrated that ginseng can impede uptake of GABA, glutamate, dopamine, noradrenalin and serotonin in rat brain synaptosomes (Tsang et al. 1985) and
that individual ginsenosides compete with agonists for binding to receptors GABAa and GABAb (Kimura et al. 1994).

1.6.5 **Anticancer Activities**

While advances in cancer therapies such as radiotherapy, chemotherapy and surgical removal are steadily improving treatment and cancer survival rates, cancer patients are increasingly turning to natural products for assistance in treating their disease, which has stimulated research investigating the chemopreventive effects of natural compounds (Park et al. 2005). Ginseng is a very effective anti-carcinogenic agent in various different ways. To begin, ginsenoside Rh2 inhibits the growth and colony forming ability of Morris hepatoma cells, thus transforming the cells characteristics back to those resembling normal liver cells both functionally and morphologically (Odashima et al. 1989). Rh2 and Rg3 also prevent prostate cancer cell proliferation (Kim et al. 2004). Specific ginsenosides also have anti-metastatic effects. Most cancer patients do not succumb to primary tumors but to metastases, which are tumor colonies that detach themselves and travel to distant regions of the body (Friedberg 1986). Rg3 was reported to restrict lung metastasis of tumor cells by preventing invasion and adhesion of these cells, as well as having anti-angiogenic properties (Mochizuki et al. 1995). Rb2 can inhibit invasion to the basement membrane by suppressing MMP-2 in some endometrial cancers and has been used as a medication to reduce secondary spreading of uterine endometrial cancers (Fujimoto et al. 2001). As demonstrated, ginseng has been thoroughly investigated and its preventative effects against carcinogenesis are extensive.
Administration of Korean red ginseng greatly reduced the incidence and proliferation of tumors induced by 7,12-dimethylbenz(a)anthracene (DMBA), urethane and aflatoxin B1 (Yun et al. 1983). Furthermore, Korean ginseng was characterized as a non-organ specific agent with protective effects against cancer mostly due to ginsenosides Rg3, Rg5 and Rh2 (Yun 2003). It also suppressed liver (Wu et al. 2001) and colon carcinogenesis through inhibition of cell proliferation (Fukushima et al. 2001). On another note, American ginseng plays a protective role against breast cancer (Duda et al. 2001) and is often taken along with breast cancer therapies due to their synergistic capabilities of suppressing breast cancer cell growth (Duda et al. 1999). Finally, orally administered ginsenoside Rh2 is associated with tumor growth retardation in human ovarian cancer cells (HRA) (Tode et al. 1993) and apoptosis induction in tumors. Rh2 increases natural killer activity in spleen cells of tumor-bearing nude mice (Nakata et al. 1998). Thus apoptosis, inhibition of cell growth and invasion, and tumor proliferation reduction seem to be some of ginseng’s many anti-carcinogenic mechanisms.

1.7 Effects of Ginseng on the Reproductive System

Research to date has indicated that ginseng’s effects on the body can be either beneficial or harmful, and its influences on the reproductive system are no exception. For example, Ginsenoside Rh2 action is associated with tumor growth retardation and apoptosis induction in ovarian cancer cells (Tode et al. 1993). Also, Ginseng may protect mouse embryos from oxidative stress via activation of embryonic antioxidant enzyme defense systems (Lee et al. 2009). In regards to
fertility, ginsenoside Re significantly enhances both fertile and infertile sperm motility in a concentration dependent manner (Zhang et al. 2006). Furthermore, Lee et al. 2008 demonstrated that treatment of early rat embryos with individual ginsenosides caused either no change in their morphological score, or improved them when compared to those cultured in a normal environment. Conflictingly, it was determined that similar treatment with individual ginsenosides actually lowered morphological scores for in vitro treated rat and mouse embryos (Chan et al. 2003, 2004; Liu et al. 2005a, 2005b, 2006), which could be potentially hazardous for embryonic and fetal development. On another note, very recent in vivo studies have demonstrated that when administering Korean Red Ginseng extract to females for two weeks before mating and throughout gestation, that maternal weight, feeding, embryonic implantation and fetal growth were not significantly affected (Shin et al. 2010). Thus the potential for ginseng to affect the reproductive system is significant and must be considered when deciding to incorporate ginseng as part of a healthy lifestyle. Additionally, with more people taking herbal supplements (Eisenberg et al. 1998) and the majority being women (MacLennan et al. 1996; Eisenberg et al. 1998; Ernst and White 2000), understanding ginseng’s effect on fertility, pregnancy and post-partum development is of utmost importance for future reproductive health. To date, no studies have investigated the effects of North American Ginseng extracts on reproductive function, and my study is focused on providing essential data related to the safety of ginseng consumption on early development in vitro and pregnancy and postpartum development in the mouse.
1.8 Hypothesis and Objectives

The principal hypothesis of this study is that aqueous and alcoholic ginseng treatment will promote preimplantation development, pregnancy and post-partum development in the mouse.

This study has addressed the following objectives to test my principal hypothesis:

1) Investigate the concentration response effect of aqueous and alcohol ginseng extracts, specific ginsenosides and polysaccharides on mouse preimplantation development in vitro.

2) Investigate the combinatorial effect of ginsenosides Rb1, Rg1 and Re on mouse preimplantation development in vitro.

3) Investigate the safety of consuming both ginseng extracts on fertility, pregnancy and post-partum development in the mouse.
Methods and Materials

2.1 Objective One

Investigate the concentration response effect of aqueous and alcoholic ginseng extracts, specific ginsenosides and polysaccharides on mouse preimplantation development.

Chemicals

KSOMa (potassium simplex optimized medium with amino acids) was used for embryo culture medium (Chemicon, Temecula, CA, USA). The Ontario Ginseng Growers Association provided four-year-old North American ginseng roots, which were shipped to Naturex (USA) for extraction. To begin, four kg of the ginseng root was ground to ½ and ¼ inch, and used to produce the AQ and ALC extract. The ground roots were soaked three times for five hours in 16 L of either water or ethanol/water (75/25, v/v) solution at 40°C. The solution was then extracted and filtered, and the excess solvent was removed by rotary evaporator under vacuum at 45°C. The three pools were combined and concentrated until the total dry solids were around 60%. Finally these concentrates were lyophilized at -50°C (Labconco, USA) under reduced pressure to produce either the AQ or ALC extracts. Yield of the powder extracts from the concentrates was about 66%. Final yields for the aqueous and alcoholic extract (mean ± standard deviation of % extractive) were 41.74 ± 4.92 and 35.30 ± 5.01 respectively. The lyophilized ALC, AQ ginseng extracts and polysaccharide fraction were obtained from Dr.
Edmund Lui and prepared in accordance with Azike et al., 2011. Ginsenosides Rb1, Rg1 and Re were purchased from Sigma (St Louis Mo. USA).

Animals

All animal care and handling procedures were conducted as approved by the University of Western Ontario, Animal Care and Veterinary Science (ACVS), standard operating procedures, that conform to guidelines produced by the Canadian Council for Animal Care (CCAC). Six-eight week old female MFI mice were purchased from Harlan Sciences, (Indiana, USA) and CD1 mice were purchased from Charles River (Canada). Each female was injected with 0.1 ml PMSG, then 0.1 ml hCG 48 h later and placed with CD1 males for mating. Pregnancy was confirmed the following morning by the presence of a vaginal plug which was considered gestational day one. On gestational day two, the mice were euthanized employing CO2 asphyxiation, followed by cervical dislocation as outlined in Western University ACVS standard operating procedures. Mice were then dissected and oviducts were removed and flushed with M2 flushing medium (Sigma, St Louis, MO, USA) to collect two-cell stage zygotes for experimental purposes.

Experimental Groups

Alcohol and Aqueous Ginseng Extracts

The two-cell embryos were washed three consecutive times in KSOMaa. Then 0.01 g of the alcoholic and aqueous ginseng extracts were individually mixed with 1 mL KSOMaa to prepare two individual 10 000 µg/ml stock solutions. These
stock solutions were then used to make five different concentrations of each extract: 0 (control), 10, 50, 250 and 1000 µg/ml. In all groups, 20 embryos were cultured in 20 µl drops under oil. All treatment groups were then put into culture at 37°C under a 5% O₂, 5% CO₂ and 90% N₂ atmosphere for 48 h.

Ginsenoside Rb1, Rg1 and, Re

Five groups were established for Rb1, Rg1, and Re. Ginsenoside concentrations were calculated to equal the actual individual concentrations in the aqueous and alcoholic extracts. 1 mg of ginsenoside Rb1, Rg1 and Re were individually mixed with 1 mL KSOMaa to prepare three -1000 µg/ml stock solutions. The stock solutions were then used to make five different concentrations for each individual ginsenoside equal to the AQ extract: Rb1 was 66.2, 16.6, 3.3, 0.7 and 0 µg/ml; Rg1 was 13.1, 2.6, 0.66, 0.15 and 0 µg/ml; Re was 55.9, 14.0, 2.8, 0.6 and 0 µg/ml, and five different concentrations for each individual ginsenoside equal to the alcoholic extract: Rb1 was 164.5, 41.1, 8.2, 1.6 and 0 µg/ml; Rg1 was 2.66, 0.66, 0.15, 0.03 and 0 µg/ml; Re was 89.6, 22.4, 4.5, 0.9 and 0 µg/ml. In all groups, 20 embryos were cultured in 20 µl drops under oil. All treatment groups were then put into culture at 37°C under a 5% O₂, 5% CO₂ and 90% N₂ atmosphere for 48 h.

Polysaccharide Fraction

For all experiments conducted with the polysaccharide fraction, CD1 females were used. Five different groups were established for treatment with the polysaccharide fraction (PSF). 15% of the aqueous extract is composed of PS,
thus this percentage was used in order to calculate the five concentrations used. The highest concentration of the aqueous extract is 1000 µg/ml, which translates into 150 µg/ml PS present in this solution at the highest concentration. 0.01 g of the PSF was mixed with 1 mL KSOMaa to prepare a 10 000 µg/ml stock solution. The stock solution was then used to make the following concentrations: 150, 37.5, 7.5, 1.5 and 0 µg/ml. In all groups, 20 embryos were cultured in 20 µl drops under oil. All treatment groups were then put into culture at 37 °C under a 5% O₂, 5% CO₂ and 90% N₂ atmosphere for 48h.

**Morphological Assessment**

Embryo development was assessed after 48 h of treatment. After treatment and culture, the cell number of each embryo was determined and it was categorized into 2, 4 or 8 cells, 8-cell compacted, morula or blastocyst groups. Micrographs of the five groups were taken and the cultures were returned to the incubator.

**Recovery from Treatment**

After the morphological assessment following 48 h of treatment, embryos were washed three consecutive times in KSOMaa and returned to culture in clean drops of culture media for another 72 h in order to determine if recovery from treatment effects was possible. After 24 and 72 h, embryos were once again categorized into their specific developmental stages and micrographs were taken. Progression on to the blastocyst stage was considered to represent full recovery from treatment.
**Statistical Analysis**

All statistical analyses were done with GraphPad Prism version 4.0c software.

Each experiment was repeated a minimum of three times using embryos collected from replicate groups of mice. Data were presented as the mean ± standard deviation (SD) for each treatment and each experiment. Two-way RM ANOVA evaluated data sets with multiple comparisons. $P < 0.05$ was considered to be statistically significant.

2.2 **Objective Two**

To investigate the combinatorial effect of ginsenosides Rb1, Rg1 and Re on mouse preimplantation development *in vitro*.

**Chemicals**

KSOMaa (potassium simplex optimized medium with amino acids) was used for embryo culture medium (Chemicon, Temecula, CA, USA). Ginsenosides Rb1, Rg1 and Re were purchased from Sigma (St Louis Mo. USA).

**Animals**

Female CD1 mice (six-eight weeks old) were purchased from Charles River (Canada). The experimental design used in objective one was also employed for the combinatorial ginsenoside experiments.
Experimental Groups

The two-cell embryos were washed three consecutive times in KSOMaa. Ginsenoside concentrations were calculated to equal the actual individual concentrations of Rb1, Rg1 and Re found in the alcoholic extract. 1 mg of purified ginsenoside Rb1, Rg1 and Re were individually mixed with 1 mL KSOMaa to prepare three -1000 µg/ml stock solutions. The stock solutions were then used to prepare a solution for each individual ginsenoside equal to three times the concentration found in 1000 µg/ml of the alcoholic extract: Rb1 was 493.5 µg/ml; Rg1 was 7.98 µg/ml; Re was 269.7 0 µg/ml. 0.5 mL of each solution was combined to give a combinatorial mixture of Rb1, Rg1 and Re equal to 257.1 µg/ml, which was then diluted to provide the five treatment concentrations of 257.1, 64.3, 12.9, 2.6 and 0 µg/ml. In all groups, 20 embryos were cultured in 20 µl drops under oil. All treatment groups were then put into culture at 37°C under a 5% O₂, 5% CO₂ and 90% N₂ atmosphere for 48 h.

The morphological assessment, embryonic recovery and statistical analyses used in objective one were repeated for the combinatorial ginsenoside experiments.

2.3 Objective Three

To investigate the safety of consuming both ginseng extracts on pregnancy and post-partum development in the mouse.
Chemicals

The AQ and ALC ginseng extracts used were provided by Dr. Edmund Lui’s lab and prepared according to Azike et al. 2011.

Animals

Female MFI mice (six-eight weeks old) were purchased from Harlan Sciences (Indiana, USA). Females receiving sham, AQ or ALC treatments were gavaged (using standard operating procedures as outlined by Western University’s ACVS) once per day at 9:00 am for 14 days prior to mating, throughout mating and during gestation. After 14 days of treatment, females were mated with CD1 males. Pregnancy was confirmed by the presence of a vaginal plug where females were then separated from males and placed into individual cages. If pregnancy was not confirmed within five days, females were asphyxiated with CO$_2$, followed by cervical dislocation as outlined above.

Gavaging

Using ACVS standard operating procedures, mice were gavaged with a straight stainless steel animal feeding needle (24G x 1 inch) attached to a 1 mL syringe. The mouse was lifted and restrained by grasping the loose skin along the nape of the neck and back with the thumb and forefinger. In order to determine how far to insert the needle, the distance from the oral cavity and the end of the xyphoid process was estimated with the feeding needle on the outside of the animal. The needle was then inserted into the right lateral side of the oral cavity and gently inserted into the back of the oral cavity. Gravity alone was used to move the
needle down the esophagus; the mouse then swallowed the needle. The fluid was then injected slowly, and the needle was gently removed. The solution volume administered was calculated in order to assure that no more than 2 mL/100 g body weight was gavaged.

**Experimental Groups**

In order to prepare the ginseng solutions, 1 mL of distilled water was mixed with 10 mg of the AQ ginseng extract. The same process was followed for the ALC ginseng extract. When administering a dose of 50 mg/kg, the gavaged volume was calculated according to individual mouse weights: \(0.05 \times \text{(weight of mouse)} / 10\). When administering a dose of 500 mg/kg, the solutions were prepared 10 times more concentrated (100 mg of each extract was used with 1 mL distilled water). Each experiment consisted of four treatment groups: control-not gavaged, sham-gavaged water, gavaged aqueous extract and gavaged alcoholic extract. Two principal experiments were conducted, females gavaged 50 mg/kg for 14 days prior to mating and throughout gestation, and females gavaged 500 mg/kg for 14 days prior to mating and throughout gestation. N values were 3-6 pregnant mice per treatment group per experiment.

**Morphological Assessment**

Once litters were born, day of birth, litter size, and gestation period were determined for each group in each experiment. Pups were also individually weighed every four days until weaning on post-partum day 20. When pups were weaned, the sex of the mice was determined.
**Statistical Analysis**

All statistical analyses were done with GraphPad Prism version 4.0c software. Data were presented as the mean ± standard deviation (SD). For litter size, gestation period and pup sex, statistical analyses were conducted with an unpaired t test and the n value was 3-6. For pup weight, two-way RM ANOVA evaluated data sets with multiple comparisons and n values ranged between 201-247. \( P < 0.05 \) was considered to be statistically significant.
Results

3.1 Concentration Response Effects of North American Ginseng Extracts and their Individual Active Compounds on Mouse Preimplantation Development in Vitro.

3.1.1 The Effect of Aqueous and Alcoholic Ginseng Extract Treatment on Preimplantation Development.

The effect of North American AQ and ALC ginseng extracts on preimplantation development in vitro was evaluated by collecting two-cell embryos from superovulated and inseminated MF1 females, and placing embryos into culture for 48 hours in 0 (control), 10, 50, 250 and 1000 µg/ml of both extracts. AQ ginseng extract treatment resulted in a concentration responsive inhibition of development (Figure 3.1A). As the concentration of ginseng increased, the numbers of embryos developing to the morula and blastocyst stages were significantly reduced (p<0.05) with development increasingly blocking at the two-cell and four-cell stages (figure 3.1A and 3.2A, C, E, G, I). With the highest concentration of 1000 µg/ml, only 1.5% of embryos developed to the morula stage, whereas 71.7% and 24.1% of control embryos progressed to the morula and blastocyst stage consecutively after 48 hours of culture. Treatment with the ALC extract presented a similar outcome with lower concentrations of 10, 50 and 250 µg/ml, significantly (p<0.05) increasing the numbers of embryos blocked in development at the two-cell stage. Interestingly, treatment with 1000 µg/ml resulted in no significant impairment of embryo development when compared to the control (figure 3.1B and 3. A, D, F, H, J).
**Figure 3.1 Development of two-cell Mouse Embryos after 48 h Treatment with Aqueous and Alcoholic Ginseng Extracts.** Two-cell embryos were flushed from the oviduct and placed into culture with the AQ (A) and ALC (B) ginseng extract for 48 hours. Treatment groups for both extracts consisted of a KSOMaa control (0 µg/ml), 10, 50, 250 and 1000 µg/ml and were put into culture at 37 °C under a 5% O₂, 5% CO₂ and 90% N₂. Embryonic development was evaluated after 48 hours and embryos were categorized as being 2-cell, 4-cell, 8-cell, 8-cell compacted, morula or blastocysts. Variations in embryo development between treatment groups are presented as the mean ± SD, representative of three independent replicates. Significant differences are represented by different superscripts (P≤0.05).
A  48 h treatment with aqueous extract

B  48 h treatment with alcoholic extract
Figure 3.2  Morphology of Embryos Following Treatment with Five different Concentrations of Aqueous and Alcoholic Ginseng Extract for 48 h. Comparative morphology of representative groups of two-cell embryos after treatment with 0 µg/ml (A, B), 10 µg/ml (C, D), 50 µg/ml (E, F), 250 µg/ml (G, H) and 1000 µg/ml (I, J) of AQ ginseng extract (A, C, E, G, I) and ALC ginseng extract (B, D, F, H, J) for 48 hours. Scale bars represent 100 µm.
Embryonic Development

Aqueous Extract 48 h in culture

Alcoholic Extract 48 h in culture

0 µg/mL

10 µg/mL

50 µg/mL

250 µg/mL

1000 µg/mL
Following treatment with AQ and ALC ginseng extracts for 48 hours, embryos were washed three consecutive times in fresh KSOMaa and returned to culture in clean drops of culture medium for an additional 72 hours to determine whether ginseng treatment effects were permanent or reversible. Measuring progression to the blastocyst stage between control and treatment groups assessed recovery from treatment. Embryos treated with 10 µg/ml of the AQ extract displayed the strongest recovery from treatment as blastocyst formation jumped from 24.1% at 0 h recovery time to 93.8% blastocysts after 72 h of recovery time. All other AQ treatment groups displayed a significant (p<0.05) decline in embryo development to the blastocyst stage when compared with the control, indicative of extremely low recovery potential (Figure 3.3A and 3.4A, B, E, F, I, J, M, N, Q, R). In contrast, embryos treated with 10, 50 and 1000 µg/ml of the ALC extract were able to fully recover from treatment by displaying no significant difference in blastocyst formation between the control and ALC treated embryos after a 72 hour recovery period. Interestingly, the developmental blockade resulting from treatment with 250 µg/ml ALC extract was only marginally reversed with 29.4% of treated embryos developing to the blastocyst stage, as opposed to 97.1% for control after 72 h of recovery time (Figure 3.3B and 3.4C, D, G, H, K, L, O, P, S, T). The difference in blastocyst formation between these two groups was significantly different (p<0.05). All data shown in Figures 3.1 and 3.3 are presented as the mean ± SD, representative of three independent replicates.

Thus both ginseng extracts negatively affect development to the blastocyst stage in vitro. The effects of AQ and ALC treatments on development were only
reversible following low concentration treatments ie 10 ug/ml for AQ extract and 10 and 50 ug/ml for ALC. Interestingly, treatment with 1000 ug/ml ALC did not negatively affect development to the blastocyst stage.
Figure 3.3  Percentage of Mouse Embryos that Recovered from Ginseng Treatment. Embryos treated for 48 h with AQ (A) and ALC (B) ginseng extracts were washed three consecutive times in KSOMaa and returned to culture in clean drops of culture media for 72 h. Progression to the blastocyst stage was considered to represent full recovery from treatment. Differences in embryo development to the blastocyst stage between treatment groups are presented as the mean ± SD, representative of three independent replicates. Significant differences are represented by different superscripts (P≤0.05).
A 72 h Recovery with Aqueous Extract

B 72 h Recovery with Alcoholic Extract
Figure 3.4  Morphology of Mouse Embryos Following Recovery from Treatment with Aqueous and Alcoholic Ginseng Extracts. Comparative morphology of representative groups of embryos following wash out and recovery from ginseng treatment with 0 µg/ml (A, B, C, D), 10 µg/ml (E, F, G, H), 50 µg/ml (I, J, K, L), 250 µg/ml (M, N, O, P) and 1000 µg/ml (Q, R, S, T) of aqueous ginseng extract (A, B, E, F, I, J, M, N, Q, R) and alcoholic ginseng extract (C, D, G, H, K, L, O, P, S, T) for 24 (A, E, I, M, Q; C, G, K, O, S) and 72 hours (B, F, J, N, R; D, H, L, P, T). Scale bars represent 100 µm.
Aqueous Extract 24 h in culture
Aqueous Extract 72 h in culture
Alcoholic Extract 24 h in culture
Alcoholic Extract 72 h in culture

Blastocyst Development
3.1.2 The Effect of Treatment with 2000 µg/ml of Aqueous Ginseng Extract on Preimplantation Development

Since the AQ ginseng extract has half the ginsenoside concentration as the ALC extract, specifically 138.7 mg/g versus 282.5 mg/g, I next investigated whether doubling the concentration of the 1000 µg/ml of AQ extract to 2000 µg/ml would replicate the outcomes observed with 1000 µg/ml of ALC ginseng extract. Two-cell embryos from MF1 females were cultured for 48 hours in 0 (control) and 2000 µg/ml of AQ extract. In contrast to my observations with using 1000 µg/ml ALC treatment (above), none of the embryos treated with 2000 µg/ml developed to the morula or blastocyst stages, and all were halted at the two-cell and four-cell stages (Figure 3.5A). Furthermore, significant (p < 0.05) differences in blastocyst formation were observed between control and treated embryos once the AQ extract treatment was washed out and the cleaned embryos returned to culture for 72 h (Figure 3.5B). All data in Figure 3.5 are presented as the mean ± SD, representative of three independent replicates. Thus these outcomes suggest that the differences in treatment effects between high concentrations of AQ and ALC on development to the blastocyst stage are unlikely to be due to differences in ginsenoside concentrations between the two extracts.
Figure 3.5 Development and Recovery of Two-cell Mouse Embryos Treated with 2000 µg/ml of Aqueous Ginseng Extract. Two-cell embryos were flushed from the oviduct and placed into culture with aqueous ginseng extract. Treatment groups consisted of a KSOMaa control (0 µg/ml) and 2000 µg/ml of the AQ extract, and were cultured at 37°C under a 5% O₂, 5% CO₂ and 90% N₂ atmosphere for 48 h (A). Embryos were then washed three consecutive times in KSOMaa and returned to culture in clean drops of culture media for 72 h (B). Embryos were categorized as being 2-cell, 4-cell, 8-cell, 8-cell compacted, morula or blastocyst after all time points. Progression on to the blastocyst stage was considered to represent full recovery from treatment. Differences in embryo development to differing embryonic stages or blastocyst formation between treatment groups are presented as the mean ± SD, representative of three independent replicates. Significant differences are represented by different superscripts (P≤0.05).
A  48 h Treatment with Aqueous Extract

B  72 h Recovery with Aqueous Extract
3.1.3 The Effect of Individual Ginsenoside Rb1 Treatment on Preimplantation Development.

My next experiments focused on attempting to define the primary constituents of the ginseng extracts that were impeding preimplantation development in vitro. I began this process by investigating the concentration response effects of increasing individual ginsenosides Rb1, Rg1 and Re concentrations on preimplantation development in vitro and concluded these experiments by investigating the effects of the polysaccharide fraction on early development.

First, the effect of ginsenoside Rb1 on preimplantation development in vitro was evaluated. Two-cell embryos from MF1 females were cultured for 48 h with concentrations of ginsenoside Rb1 equal to those found in the aqueous extract (Figure 3.6A, B) and alcoholic extract (figure 3.6C, D). AQ level treatment groups consisted of 0, 0.7, 3.3, 16.6 and 66.2 µg/ml of Rb1. In contrast to the treatment outcomes with the AQ extracts, treatment with Rb1 alone did not significantly block embryo development at the two-cell or four-cell stages. However, there was a significant difference (p<0.05) between the control and treatment with 16.6 µg/ml Rb1, with the treated group displaying a higher percentage of morula. This was accompanied by the observation that no embryos in the treated groups developed to the blastocyst stage after 48 h treatment, while the control group displayed a 10% blastocyst formation frequency (Figure 3.6A and 3.7A-E). Experimentation using ALC Rb1 treatment levels (0, 1.6, 8.2, 41.1 and 164.5 µg/ml) resulted in a significant variation between the control and treatment with 8.2 µg/ml of ginsenoside Rb1 (Figure 3.6C). 84.1% of embryos in
the control group compared to only 65.6% of embryos treated with 8.2 µg/ml progressed to the morula stage. No other significant differences in embryo development were observed between the treatment groups (Figure 3.6C and 3.7F-J).

Embryos treated for 48 h with ginsenoside Rb1 were washed out in KSOMaa and returned to culture in clean drops of culture media for 72 h. Progression to the blastocyst stage was considered to represent full recovery from treatment. For both Rb1 experiments (Figure 3.6B and 3.8A-J; Figure 3.6D and 3.8K-T), while blastocyst formation was low at the 0 h mark, embryos in all treatment groups were able to recover from treatment and no significant differences in development were observed between any treatment groups after 72 h in culture. All data shown in figure 3.6 is presented as the mean ± SD, representative of three independent replicates.
Figure 3.6 Development and Recovery of Mouse Embryos Following Treatment with Ginsenoside Rb1. Two-cell embryos were flushed from the oviduct and placed into culture for 48 h with concentrations of ginsenoside Rb equal to those found in the aqueous (A) and alcoholic (C) extracts. Treatment groups consisted of: 0, 0.7, 3.3, 16.6 and 66.2 µg/ml (A, B); 0, 1.6, 8.2, 41.1 and 164.5 µg/ml (C, D). Embryos were then washed three consecutive times in KSOMaa and returned to culture in clean drops of culture media for 72 h (B, D). Differences in embryo development to differing embryonic stages or blastocyst formation between treatment groups are presented as the mean ± SD, representative of three independent replicates. Significant differences are represented by different superscripts (P≤0.05).
A 48 h Treatment with Rb1

B 72 h Recovery with Rb1

C 48 h Treatment with Rb1

D 72 h Recovery with Rb1

Embryonic Stage

Blastocyst Development
Figure 3.7  Morphology of Mouse Embryos Following Rb1 Treatment.

Comparative morphology of representative groups of embryos after 48 h treatment with concentrations of Rb1 equal to those found in the aqueous (A-E) and alcoholic (F-J) ginseng extracts. Treatment groups consisted of: 0 (A), 0.7 (B), 3.3 (C), 16.6 (D) and 66.2 µg/ml (E); 0 (F), 1.6 (G), 8.2 (H), 41.1 (I) and 164.5 µg/ml (J). Scale bars represent 100 µm.
Ginsenoside Rb1

48 h in culture

Embryonic Development
Figure 3.8  Morphology of Mouse Embryos Following Recovery from Rb1 Treatment. Comparative morphology of representative groups of embryos following wash out and recovery from treatment with 0 µg/ml (A, B and K, L), 0.7 µg/ml (C, D), 3.3 µg/ml (E, F), 16.6 µg/ml (G, H), 66.2 µg/ml (I, J), 1.6 µg/ml (M, N), 8.2 µg/ml (O, P), 41.1 µg/ml (Q, R) and 164.5 µg/ml (S, T) of ginsenoside Rb1 for 24 (A-J) and 72 hours (K-T). Scale bars represent 100 µm.
Ginsenoside Rb1

24 h in culture 72 h in culture

A   B  0 μg/mL
C   D  0.7 μg/mL
E   F  3.3 μg/mL
G   H  16.6 μg/mL
I   J  66.2 μg/mL

Ginsenoside Rb1

24 h in culture 72 h in culture

K   L  0 μg/mL
M   N  1.6 μg/mL
O   P  8.2 μg/mL
Q   R  41.4 μg/mL
S   T  164.5 μg/mL

Blastocyst Development
3.1.4 The Effect of Individual Ginsenoside Rg1 Treatment on Preimplantation Development.

Two-cell embryos from MF1 females were cultured for 48 h with concentrations of ginsenoside Rg1 equal to those found in the aqueous extract (Figure 3.9A, B) and alcoholic extract (Figure 3.9C, D). AQ levels consisted of 0, 0.13, 0.66, 2.66 and 13.1 µg/ml. Treatment with Rg1 at AQ levels for 48 h resulted in no significant differences (p< 0.05) in embryonic development between any of the treatment groups (Figure 3.9A and 3.10A-E). ALC Rg1 treatment group levels included 0, 0.03, 0.15, 0.66 and 2.66 µg/ml. The most notable difference in developmental outcomes occurred between the control group, where 90.5% of embryos progressed to the morula stage whereas in the lowest concentration treatment of 0.03 µg/ml, only 71% of the embryos treated with Rg1 progressed to the morula stage. In addition, development to the morula stage varied significantly (p<0.05) between treatment with concentrations of 0.03, 0.66 and 2.66 µg/ml as well as 0.66 and 2.66 µg/ml of ginsenoside Rg1 (Figure 3.9C and 3.10F-J).

Embryos treated for 48 h with ginsenoside Rg1 were then washed three consecutive times in KSOMaa and returned to culture in clean drops of culture media for 72 h. No significant differences in blastocyst development were observed between controls, and any of the AQ Rg1 treatment groups (Figure 3.9B and Figure 3.11A-J). In contrast, within the ALC Rg1 treatment groups, blastocyst formation was delayed for the 0.03 and 0.15 µg/ml Rg1 treatments in comparison to the control at the 24 h time point. However, after 72 h in culture, no significant differences in blastocyst development between groups were
observed (figure 3.9D and 3.11K-T). All data shown in Figure 3.9 is presented as the mean ± SD, representative of three independent replicates.
Figure 3.9  Development and Recovery of Mouse Embryos Following Treatment with Ginsenoside Rg1. Two-cell embryos were flushed from the oviduct and placed into culture for 48 h with concentrations of ginsenoside Rg1 equal to those found in the aqueous (A) and alcoholic (C) extracts. Treatment groups consisted of: 0, 0.13, 0.66, 2.66 and 13.1 µg/ml (A, B); 0, 0.03, 0.15, 0.66 and 2.6 µg/ml (C, D). Embryos were then washed three consecutive times in KSOMaa and returned to culture in clean drops of culture media for 72 h (B, D). Embryos were categorized as being 2-cell, 4-cell, 8-cell, 8-cell compacted, morula or blastocyst after all time points. Progression on to the blastocyst stage was considered to represent full recovery from treatment. Differences in embryo development to differing embryonic stages or blastocyst formation between treatment groups are presented as the mean ± SD, representative of three independent replicates. Significant differences are represented by different superscripts (P≤0.05).
**Figure 3.10  Morphology of Mouse Embryos Following Rg1 Treatment.**

Comparative morphology of representative groups of embryos after 48 h treatment with concentrations of Rg1 equal to those found in the aqueous (A-E) and alcoholic (F-J) ginseng extracts. Treatment groups consisted of: 0 (A), 0.13 (B), 0.66 (C), 2.66 (D) and 13.1 µg/ml (E); 0 (F), 0.03 (G), 0.15 (H), 0.66 (I) and 2.6 µg/ml (J). Scale bars represent 100 µm.
Embryonic Development

Ginsenoside Rg1
48 h in culture

A
0 μg/mL

0.13 μg/mL

B

0.03 μg/mL

C
0.66 μg/mL

0.15 μg/mL

D
2.66 μg/mL

0.66 μg/mL

E
13.1 μg/mL

2.6 μg/mL

F
0 μg/mL

G

H

I

J
Figure 3.11  Morphology of Mouse Embryos Following Recovery from Rg1 Treatment. Comparative morphology of representative groups of embryos once recovered from treatment with 0 µg/ml (A, B and K, L), 0.13 µg/ml (C, D), 0.66 µg/ml (E, F), 2.66 µg/ml (G, H), 13.1 µg/ml (I, J), 0.03 µg/ml (M, N), 0.15 µg/ml (O, P), 0.66 µg/ml (Q, R) and 2.6 µg/ml (S, T) of ginsenoside Rg1 for 24 (A-J) and 72 hours (K-T). Scale bars represent 100 µm.
Blastocyst Development
3.1.5 The Effect of Individual Ginsenoside Re Treatment on Preimplantation Development.

Two-cell embryos from MF1 females were cultured for 48 h with concentrations of ginsenoside Re equal to those found in the aqueous extract (Figure 3.12A, B) and the alcoholic extract (Figure 3.12C, D). Treatment groups consisted of: 0, 0.6, 2.8, 14.0 and 55.9 µg/ml for AQ Re levels and 0, 0.9, 4.5, 22.4 and 89.9 µg/ml for ALC Re levels. For all treatments, including 72 h recovery, with both AQ Re and ALC Re concentrations, no significant variations in embryo development between the control and treatment groups were observed (Figure 3.12A,C and 3.13; Figure 3.12B and 3.14A-J; Figure 3.12D and 3.14K-T).
Figure 3.12 Development and Recovery of Mouse Embryos Following Treatment with Ginsenoside Re. Two-cell embryos were flushed from the oviduct and placed into culture for 48 h with concentrations of ginsenoside Re equal to those found in the aqueous (A) and alcoholic (C) extracts. Treatment groups consisted of: 0, 0.6, 2.8, 14 and 55.9 µg/ml (A, B); 0, 0.9, 4.5, 22.4 and 89.9 µg/ml (C, D). Embryos were then washed three consecutive times in KSOMaa and returned to culture in clean drops of culture media for 72 h (B, D). Embryos were categorized as being 2-cell, 4-cell, 8-cell, 8-cell compacted, morula or blastocyst after all time points. Progression on to the blastocyst stage was considered to represent full recovery from treatment. No significant differences were observed between treatment groups.
A 48 h Treatment with Re

B 72 h Recovery with Re

C 48 h Treatment with Re

D 72 h Recovery with Re

Embryonic Stage

Blastocyst Development

Percentage (%)
Figure 3.13  Morphology of Mouse Embryos Following Re Treatment.

Comparative morphology of representative groups of embryos after 48 h treatment with concentrations of Re equal to those found in the aqueous (A-E) and alcoholic (F-J) ginseng extracts. Treatment groups consisted of: 0 (A), 0.6 (B), 2.8 (C), 14.0 (D) and 55.9 µg/ml (E); 0 (F), 0.9 (G), 4.5 (H), 22.4 (I) and 89.6 µg/ml (J). Scale bars represent 100 µm.
Ginsenoside Re

48 h in culture

Embryonic Development
Figure 3.14 Morphology of Mouse Embryos Following Recovery from Re Treatment. Comparative morphology of representative groups of embryos following recovery period with 0 µg/ml (A, B and K, L), 0.6 µg/ml (C, D), 2.8 µg/ml (E, F), 14.0 µg/ml (G, H), 55.9 µg/ml (I, J), 0.9 µg/ml (M, N), 4.5 µg/ml (O, P), 22.4 µg/ml (Q, R) and 89.6 µg/ml (S, T) of ginsenoside Re for 24 (A-J) and 72 hours (K-T). Scale bars represent 100 µm.
### Blastocyst Development

<table>
<thead>
<tr>
<th>Concentration (μg/mL)</th>
<th>24 h in culture</th>
<th>72 h in culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>A</td>
<td>K</td>
</tr>
<tr>
<td>0.6</td>
<td>C</td>
<td>M</td>
</tr>
<tr>
<td>2.8</td>
<td>E</td>
<td>O</td>
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<tr>
<td>14.0</td>
<td>G</td>
<td>Q</td>
</tr>
<tr>
<td>55.9</td>
<td>I</td>
<td>S</td>
</tr>
</tbody>
</table>

- **Ginsenoside Re**
  - 24 h in culture
  - 72 h in culture
3.1.6 The Effect of Treatment with the Polysaccharide Fraction (PSF) on Preimplantation Development.

Two-cell embryos from CD1 females were cultured for 48 hours in 0 (control), 1.5, 7.5, 37.5 and 150 µg/ml of PSF. No significant differences in embryo development were identified when comparing preimplantation developmental progression between treatment groups (Figure 3.15A and 3.16A, D, G, J, M). Notably, after 48 h in treatment, a much higher percentage of embryos developed to the blastocyst stage for all groups than observed in all other previous experiments. This is likely due to the fact that embryos cultured with PSF were from CD1 and not MFI females that were used for all previous experiments. CDI and MFIs are both outbred lines of mice that are commonly used to generate preimplantation embryos for research purposes. We have preferred MF1s simply because they display a more robust response to hormonal superovulation and thus allow for the collection of greater numbers of oocytes and embryos from fewer mice than CD1s do. No differences in development have been reported in the literature to date for embryos derived from these lines, however we have noted that CDI embryos do develop faster in vitro than MF1 embryos. Our supplier of MF1 mice (Harlan Inc) has discontinued offering them due to the small number of labs around the world that use these mice. Thus we have had to shift exclusively to CD1 mice for all further experiments conducted in the lab.

Embryos treated for 48 h with PSF were not affected by the treatment and developed normally. Once washed 3 consecutive times in KSOMaa and returned to culture in clean drops of culture media for 72 h, natural development continued
and no significant variations in blastocyst formation were noted between any treatment groups (Figure 3.15B and 3.16B, C, E, F, H, I, K, L, N, O). All data shown in Figure 3.15 is presented as the mean ± SD, representative of three independent replicates. Thus the overall conclusion from all of the single agent treatment experiments is that no single agent replicates the collective effects of AQ or ALC extract treatment observed on preimplantation development. Thus the negative impact on development observed from AQ and ALC treatment must stem from the collective effects of several of the ginseng extract components acting on the early embryo and restricting development.
Figure 3.15 Development and Recovery of Mouse Embryos Treated with PSF. Two-cell embryos were flushed from the oviduct and placed into culture with PSF. Treatment groups consisted of KSOMaa control (0 µg/ml), 1.5, 7.5, 37.5 and 150 µg/ml and were cultured at 37°C under a 5% O₂, 5% CO₂ and 90% N₂ atmosphere for 48 h (A). Embryos were then washed three consecutive times in KSOMaa and returned to culture in clean drops of culture media for 72 h (B). Embryos were categorized as being 2-cell, 4-cell, 8-cell, 8-cell compacted, morula or blastocyst after all time points. Progression on to the blastocyst stage was considered to represent full recovery from treatment. No significant differences were observed between treatment groups.
A  48 h Treatment with Polysaccharide Fraction

- 0 µg/mL
- 1.5 µg/mL
- 7.5 µg/mL
- 37.5 µg/mL
- 150 µg/mL

Embryonic Stage

B  72 h Recovery with Polysaccharide Fraction

- 0 µg/mL
- 1.5 µg/mL
- 7.5 µg/mL
- 37.5 µg/mL
- 150 µg/mL

Blastocyst Development
Figure 3.16 Morphology of Embryos Following PSF Treatment. Comparative morphology of representative groups of mouse Two-cells after treatment for 48 h (A, D, G, J, M) and recovery for 24 h (B, E, H, K, N) and 72 h (C, F, I, L, O) with 0 µg/ml (A, B, C), 1.5 µg/ml (D, E, F), 7.5 µg/ml (G, H, I), 37.5 µg/ml (J, K, L) and 150 µg/ml (M, N, O) of PSF. Scale bars represent 100 µm.
Embryonic Development
3.2 Investigate the Combinatorial Effect of Ginsenosides Rb1, Rg1 and Re on Mouse Preimplantation Development in Vitro

Two-cell embryos from CD1 females were cultured for 48 hours in 0 (control), 2.6, 12.9, 64.3 and 257.1 µg/ml of a combinatorial solution of Rb1, Rg1 and Re. No significant differences in embryo development were identified when comparing preimplantation developmental progression between treatment groups (Figure 3.17A and 3.18A, D, G, J, M). Notably, after 48 h in treatment, a much higher percentage of embryos developed to the blastocyst stage for all groups than observed in most other previous experiments. This is likely due to the fact that embryos cultured with the combinatorial solution were from CD1 and not MF1 females that were used for the experiments outlined above. CD1 embryos simply develop faster in vitro than MF1 embryos, which accounts for the higher blastocyst formation rate.

Embryos treated for 48 h with a combination of Rb1, Rg1 and Re were not affected by the treatment, and developed normally. Embryos were then washed three consecutive times in KSOMaa and returned to culture in clean drops of culture media for 72 h. While blastocyst formation for embryos treated with 64.3 µg/ml was reduced compared to embryos treated with 12.9 µg/ml, no significant variations in blastocyst development were observed between the control and any treatment groups at any time points throughout recovery (Figure 3.17B and 3.18B, C, E, F, H, I, K, L, N, O). All data shown in Figure 3.17 is presented as the mean ± SD, representative of three independent replicates. Thus the overall conclusion from the experiment with the combinatorial ginseng solution is that the collective effects of AQ and ALC extract treatment observed on preimplantation
development are not due to this particular combination of ginsenosides. Therefore, the negative impact on development observed from AQ and ALC treatment must stem from a ginsenoside or non-ginsenoside compound that has not been investigated in this project, or the collective effects of several of the ginseng extract components acting on the early embryo and restricting development.
Figure 3.17  Development and Recovery of Mouse Embryos Treated with a Combinatorial Solution of Ginsenosides Rb1, Rg1 and Re. Two-cell embryos were flushed from the oviduct and placed into culture with a combination of Rb1, Rg1 and Re. Treatment groups consisted of KSOMaa control (0 µg/ml), 2.6, 12.9, 64.3 and 257.1 µg/ml and were cultured at 37°C under a 5% O₂, 5% CO₂ and 90% N₂ atmosphere for 48 h (A). Embryos were then washed three consecutive times in KSOMaa and returned to culture in clean drops of culture media for 72 h (B). Embryos were categorized as being 2-cell, 4-cell, 8-cell, 8-cell compacted, morula or blastocyst after all time points. Progression on to the blastocyst stage was considered to represent full recovery from treatment. No significant differences were observed between treatment groups.
A 48 h Treatment with Rb1, Rg1 and Re

B 72 h Recovery with Rb1, Rg1 and Re
Figure 3.18  Morphology of Embryos Following Treatment with a Combinatorial Solution of Ginsenosides Rb1, Rg1 and Re. Comparative morphology of representative groups of mouse two-cells after treatment for 48 h (A, D, G, J, M) and recovery for 24 h (B, E, H, K, N) and 72 h (C, F, I, L, O) with 0 µg/ml (A, B, C), 2.6 µg/ml (D, E, F), 12.9 µg/ml (G, H, I), 64.3 µg/ml (J, K, L) and 257.1 µg/ml (M, N, O) of a combinatorial solution of Rb1, Rg1 and Re. Scale bars represent 100 µm.
Rb1, Rg1 & Re
48 h in culture

Rb1, Rg1 & Re
Recovery
24 h in culture

Rb1, Rg1 & Re
Recovery
72 h in culture

0 μg/mL

2.9 μg/mL

12.9 μg/mL

64.3 μg/mL

257.1 μg/mL

Embryonic Development
3.3 Determine the Safety of consuming both Ginseng Extracts on Pregnancy and Post-Partum Development in the Mouse

The effects of treatment with AQ or ALC extract on mouse preimplantation development in vitro vary substantially from the reports of effects of treatment on mouse pregnancy and post-partum development using Red Korean ginseng. Thus it was essential to extend my studies and conduct in vivo experiments using North American ginseng to determine the effects, if any, of these extracts on pregnancy and post-partum development in the mouse.

3.3.1 Females Gavaged with 50 mg/kg of Ginseng Extracts for Two Weeks prior to Mating and throughout Gestation.

Female MF1 mice were placed into control (no gavage), sham (water gavage) and experimental treatments (gavage of 50 mg/kg of either AQ or ALC ginseng extract) each day for two weeks prior to mating and throughout gestation. 3-6 experimental replicates were carried out for each treatment group. Gestational day 0 was established by the presence of a vaginal plug after mating. Following parturition, the gestation period (average of 19 days for control) (Figure 3.19A) and litter size (average of 12.75 pups per litter for control) (Figure 3.19B) were determined. Developmental growth was then assessed and pups were weaned and sexed on post-partum day 20. The percentage of males and females in each litter was compared between different treatment groups, and the sex ratio was determined (Figure 3.19C). No significant differences were observed between treatment groups for gestation period, litter size or sex ratio.
Pup growth rate was determined by weighing 247 individual pups every four days from birth to post-partum day 20. Differences in pup weight among the treatment groups were observed beginning on post partum day four. While none of these variations in growth were significantly different among the treatment groups, they persisted until day 20 post partum (Figure 3.20A). To determine whether the differences in development observed in Figure 3.20A are due to possible variations in growth rates amid males and females, weight was recorded according to sex on day 20. There were no significant differences in male pup weight on post-partum day 20 between any treatment groups (Figure 3.20B). However, pup weight did vary significantly between the control (average of 14.1g) and ALC (average of 13.3g) groups of female pups on post-partum day 20 (Figure 3.20C). All data shown in Figure 3.19 and 3.20 are presented as the mean ± SD.
Figure 3.19  The Effect of Pre-Pregnancy and Gestational Ginseng Treatment (50 mg/kg) on Pregnancy and Post-Partum Development. Female MF1 mice were placed into control (no gavage), sham (water gavage) and experimental treatments (gavage of 50 mg/kg of either AQ or ALC ginseng extract) for two weeks prior to mating and throughout gestation. Following parturition, gestation period in days (A) and number of pups per litter (B) were determined for each female. Pups were then sexed on post-partum day 20 and the percentage of males vs. females in each litter was evaluated (C). Differences in gestation period, litter size and sex ratio between treatment groups are presented as the mean ± SD, representative of 3-6 independent replicates. No significant differences were observed between treatment groups.
A  
**Gestation for Mice Gavaged with 50 mg/kg Ginseng**

![Gestation Graph](image)

B  
**Litter Size for Mice Gavaged with 50 mg/kg Ginseng**

![Litter Size Graph](image)

C  
**Sex Ratio between Treatment Groups**

![Sex Ratio Graph](image)
Figure 3.20  The Effect of Pre-Pregnancy and Gestational Ginseng Treatment (50 mg/kg) on Post-Partum Pup Weight and Growth. Female MF1 mice were placed into control (no gavage), sham (water gavage) and experimental treatments (gavage of 50 mg/kg of either AQ or ALC ginseng extract) for 20 weeks prior to mating and throughout gestation. Following parturition, pups were individually weighed every four days until weaning on post-partum day 20 (A). Once weaned, pups were sexed and weight was recorded according to sex in order to determine differences between male (B) and female (C) developmental weight gain between treatment groups. Differences in pup growth between treatment groups are presented as the mean ± SD, representative of 49-77 (A) and 3-6 (B, C) independent replicates. Significant differences are represented by different superscripts (P≤0.05).
A) Difference in Pup Weight between Treatment Groups

B) Weight Difference in Male Pups on day 20

C) Weight Difference in Female Pups on day 20
3.3.2 Females Gavaged with 500 mg/kg of Ginseng Extracts for Two Weeks prior to Mating and throughout Gestation.

Female MF1 mice were placed into control (no gavage), sham (water gavage) and experimental treatments (gavage of 500 mg/kg of either AQ or ALC ginseng extract) each day for two weeks prior to mating and throughout gestation. 3-6 experimental replicates were carried out for each treatment group. Gestational day 0 was established by the presence of a vaginal plug after mating. Following parturition, significant (p<0.05) variations in gestation period were noted between the control and both AQ and ALC groups, with a difference of 0.78 and 1 day respectively. Furthermore, a significant difference (p<0.05) of 0.68 days was observed between the sham and ALC groups (Figure 3.21A). The average number of pups per litter between treatment groups also varied significantly (p<0.05) between the control (15.03 pups per litter) and sham (12 pups per litter) treatments, supporting our observation that gavage can influence gestation period (Figure 3.21B). No significant differences in sex ratio were detected in any of the treatment groups on day 20 post-partum (Figure 3.21C).

To evaluate pup growth and development, 212 individual pups were weighed every four days from birth to post-partum day 20. No significant differences (p<0.05) were observed in pup weight among treatment groups until post-partum day 20. On day 20 post-partum there was a significant (p<0.05) difference between the ALC treatment and the three other groups (control, sham and AQ) (Figure 3.22A). To determine whether the observed differences in weight were due to possible variations in growth between males and females, weight was determined for each sex on day 20. A significant difference (p<0.05)
in male pup weight was observed on post-partum day 20 between the sham (mean = 13.4g) and ALC (mean = 15g) groups (Figure 3.22B). However, no significant differences in female pup weight on post-partum day 20 were observed between any treatment groups (Figure 3.22C). All data shown in Figure 3.21 and 3.22 are presented as the mean ± SD. Overall the results support a cautionary approach to determining whether ginseng extract consumption has a negative influence on pregnancy and post-partum development. Gavaging certainly has major influence on gestation term and litter size, however independent effects of AQ and ALC extracts on gestation period and pup growth were also observed. In addition, the effects of ginseng consumption display some signs of gender bias, especially on weight gain.
Figure 3.21  The Effect of Pre-Pregnancy and Gestational Ginseng Treatment (500 mg/kg) on Pregnancy and Post-Partum Development.

Female MF1 mice were placed into control (no gavage), sham (water gavage) and experimental treatments (gavage of 500 mg/kg of either AQ or ALC ginseng extract) for two weeks prior to mating and throughout gestation. Following parturition, gestation period in days (A) and number of pups per litter (B) were determined for each female. Pups were then sexed on post-partum day 20 and the percentage of males vs. females in each litter was evaluated (C). Differences in gestation period, litter size and sex ratio between treatment groups are presented as the mean ± SD, representative of 3-6 independent replicates. Significant differences are represented by different superscripts (P≤0.05).
A. Gestation for Mice Gavaged with 500 mg/kg Ginseng

B. Litter Size for Mice Gavaged with 500 mg/kg Ginseng

C. Sex Ratio between Treatment Groups
Figure 3.22  The Effect of Pre-Pregnancy and Gestational Ginseng Treatment (500 mg/kg) on Post-Partum Pup Weight and Growth. Female MF1 mice were placed into control (no gavage), sham (water gavage) and experimental treatments (gavage of 500 mg/kg of either AQ or ALC ginseng extract) for two weeks prior to mating and throughout gestation. Following parturition, pups were individually weighed every four days until weaning on post-partum day 20 (A). Once weaned, pups were sexed and weight was recorded according to sex in order to determine differences between male (B) and female (C) developmental weight gain between treatment groups. Differences in pup growth between treatment groups are presented as the mean ± SD, representative of 27-66 (A) and 3-6 (B, C) independent replicates. Significant differences are represented by different superscripts (P≤0.05).
A) Difference in Pup Weight between Treatment Groups

B) Weight Difference in Male Pups on day 20

C) Weight Difference in Female Pups on day 20
Discussion

4.1 Concentration Response Effect of the Aqueous and Alcoholic Ginseng Extracts derived from North American Ginseng on Mouse Preimplantation Development in Vitro.

Ginseng is a natural herbal product and its consumption and inclusion as part of a healthy diet is on a dramatic rise worldwide. Due to the many purported health benefits associated with this herb, it is one of 10 most commonly used herbal medicines in the United States (Bent and Ko 2004). Women are the predominant users of herbal medicines (MacLennan et al. 1996; Eisenberg et al. 1998; Ernst and White 2000). In a US survey, 9.1% of pregnant women declared the use of these products during their current pregnancy (Gibson et al. 2001). In China, as high as 15% of women consumed ginseng during pregnancy with the primary reason being that it is believed to be “good for pregnancy and the fetus” (Ong et al. 2005). Due to the lack of information on the safety of ginseng consumption during pregnancy, its effects on preimplantation development, pregnancy and post-partum development were assessed in this present study. In summation, it was discovered that AQ and ALC ginseng extract treatments caused a concentration dependent inhibition of development when applied directly to preimplantation embryos in culture. Experiments were repeated with individual ginsenosides, and ginsenosides Rb1 and Rg1 reduced the number of embryos progressing to the morula stage at low concentrations. Importantly, pregnancy and post-partum development were affected with an orally administered high dose of 500 mg/kg of North American ginseng (NAG) extracts.
This study indicates that direct exposure to ginseng extracts is detrimental to the completion of normal early mammalian development.

The effect of ginseng and ginsenosides on postimplantation embryos has been examined (Chan et al. 2003, 2004; Liu et al. 2005a, 2005b, 2006; Lee et al. 2008), however its direct influence on preimplantation embryo development and thus embryo implantation, has not been investigated to date. Furthermore, most experiments determining ginseng’s effect on reproduction were undertaken with Korean or Chinese ginseng. North America is known to now produce some of the most potent forms of ginseng, and this is becoming an important agricultural crop as well as a sought after herbal medicine. It is thus essential that research investigate the safety of NAG on reproduction and pregnancy. Furthermore, due to such differences in composition between the AQ and ALC extracts (as previously described), it is important to investigate both extracts separately. In this present study, the effects of AQ and ALC extracts derived from North American ginseng, as well as the influence of its individual active compounds on preimplantation development in the mouse were assessed. Treatment with the AQ ginseng extract resulted in a concentration-dependent inhibition of development after 48 h in culture. Treatment with the lowest concentration of 10 µg/ml of AQ extract did not affect embryo development, however 50, 250 and 1000 µg/ml AQ extract levels increasingly halted development at the two-cell and four-cell stages. Furthermore, recovery potential was extremely low for these three treatments and a significant blockade of blastocyst development was observed. Treatment with the ALC extract produced similar outcomes. The main variation between treatments with the two types of extracts was observed with
the 1000 µg/ml ALC concentration. It is challenging to understand the mechanistic basis for the observed difference in outcomes using the 1000 µg/ml concentration of both ginseng extracts, however one main difference between both extracts is that the ALC extract contains twice the levels of ginsenosides as the AQ extract. We have very little understanding regarding the precise mechanism for ginseng extract action on any cell system, including the early embryo. However, my outcomes from the ALC extract experiment are reminiscent of several types of ligand/receptor interactions where high levels of ligand result in receptor desensitization and loss of ligand treatment effects. Of course this is speculation and requires a great deal more research to fully understand, but it may be possible that the highest ALC concentration did not impair development simply because of concentration effects on cell ligand/receptor signaling systems. The identity of those ligand/receptor interactions requires extensive new research to uncover them.

In addition each extract has a unique composition. For example, the AQ extract has less Rb1, Rb2, Rc, Rd and Re, has greater levels of Rg1, and is composed of 15% PS, whereas the ALC extract does not contain PS. Due to the complex composition of ginseng products, the precise compound and mechanism causing the blockade in development is not known, but certainly should become a focus for further research in this area. Thus the active ingredient halting embryonic development and causing the different outcomes observed for both extracts may not necessarily be due to a specific ginsenoside or PS but to other compounds found in the extract as well as a possible combination of certain composites. Finally, excluding embryos treated with 250 µg/ml, all other groups
treated with the ALC extract recovered from the 48 h treatment and progressed onto the blastocyst stage after 72 h recovery. The fact that embryonic development was not as severely affected by ALC treatment as that observed with the AQ extract may have provided these embryos with a better opportunity to offset any negative treatment effects and continue their normal progression to the blastocyst stage once removed from treatment.

As previously mentioned, the ALC ginseng extract has double the concentration of ginsenosides as the AQ extract. Embryos were treated with 2000 µg/ml of the AQ ginseng extract, which has the same total ginsenoside concentration as 1000 µg/ml of ALC, in order to determine whether the lack of influence imparted by ALC treatment at this concentration was due to a protective benefit of high ginsenoside levels. However, the ALC outcomes were not replicated in this experiment, and instead of observing no negative effect on development from the AQ extract, treatment effects were amplified and none of the embryos treated with 2000 µg/ml developed to the morula or blastocyst stages as all were halted at the two-cell and four-cell stages. Furthermore, none of the embryos recovered from this treatment. Taken together, these results suggest that the absence of an effect on embryonic development with 1000 µg/ml of ALC is not simply due to higher total ginsenoside concentrations, but likely due to a combination of composites or non-ginsenoside compounds distinct from that found in the AQ extract. In conclusion, the detrimental effect observed on embryonic development induced by treatment with the AQ and ALC ginseng extracts is concentration dependent, and direct embryo exposure to ginseng extracts is embryotoxic and thus detrimental to preimplantation development.
Furthermore, once removed from treatment, embryo recovery is limited if possible at all, and most embryos are not able to resume development once halted at the two-cell and four-cell stages.

4.2 Concentration Response Effect of the Polysaccharide Fraction and Individual Ginsenosides Rb1, Rg1, and Re on Mouse Preimplantation Development in Vitro.

My subsequent experiments focused on attempting to define the primary constituents of the ginseng extracts that were impeding preimplantation development in vitro. The exact compound(s) responsible for the embryonic developmental delay observed with the AQ and ALC ginseng extracts have yet to be identified due to the complexity of the ginseng plant and all the products derived from it. Due to the fact that ginsenosides are the major bioactive component in ginseng and so much research has been centered around their effects on reproduction (Chan et al. 2003, 2004; Liu et al. 2005a, 2005b, 2006; Lee et al. 2008), embryos were treated with individual ginsenosides Rb1, Rg1 and Re in order to determine their individual effect on preimplantation development. Each ginsenoside has a unique chemical structure, which is the foundation for their different pharmacological and physiological effects in the body (Sievenpiper et al. 2003). Experiments conducted with Rb1 (PD), Rg1 and Re (PT) also assured that both major ginsenoside groups, protopanaxadiols and protopanaxatriols (Tawab et al. 2010) were being tested. In addition, these ginsenosides are extensively researched due to high concentrations found in most ginseng species, including AQ and ALC extracts, which made them an ideal
starting point for assessing the primary constituents of the ginseng extracts that could be impeding preimplantation development *in vitro*.

Rb1 is the major ginsenoside found in North American Ginseng (NAG) and is representative of the PD group (Tawab et al. 2010). Previous studies have investigated the direct effect of ginsenoside Rb1 on organogenesis in postimplantation embryos, and have reported that Rb1 treatment significantly reduces the total morphological scores of rat and mouse embryos when using concentrations $\geq 30 \, \mu\text{g/ml}$. In addition, the detrimental effect on morphogenesis was concentration dependent (Chan et al. 2003; Liu et al. 2005). In my study, preimplantation embryos treated with Rb1 displayed a reduced rate of development to the morula stage when compared to the control. However this effect was not concentration dependent and was only observed with a low concentration of 8.2 $\mu\text{g/ml}$. Furthermore, all embryos recovered from treatment and progressed to the blastocyst stage.

Rg1 is found in highest concentrations in the AQ ginseng extract derived from NAG and is representative of the PT group. Liu et al. 2006 investigated the effect of Rg1 on postimplantation embryos, and found that it remarkably decreased the total morphological score of mouse and rat embryos when concentrations of 30 and 50 $\mu\text{g/ml}$ were tested. Furthermore, postimplantation embryonic growth was affected using concentrations as low as 10 $\mu\text{g/ml}$. In my present study however, the only significant difference in development was observed between the control and embryos treated with 0.03 $\mu\text{g/ml}$ of Rg1. Furthermore, while blastocyst formation was delayed for groups treated with 0.03 and 0.15 $\mu\text{g/ml}$ during recovery, all embryos recovered from treatment after 72 h
in culture. In contrast to Liu et al. 2006, the embryonic developmental delay was not concentration dependent and was only observed in the 0.03 µg/ml treatment. Future studies investigating the effects of Rg1 on early development should consider the use of higher concentration treatments. Thus my study certainly varied from the outcomes presented in the Liu et al. 2005 and 2006 studies showing effects of Rb1 and Rg1 treatment on postimplantation development, as I only observed treatment effects on preimplantation embryos using lower concentrations of either ginsenoside, and the effects were not concentration dependent. I obtained the individual Rg1 and Rb1 ginsenosides from Sigma chemical company, and it is advertised to be a highly purified form of each ginsenoside. It is possible the purity of the ginsenoside samples varied between my study and Liu et al 2005 and 2006, which may account for the variation in outcomes between the studies. Even though I observed some restriction of development with low concentrations of Rb1 and Rg1, the fact that all embryos readily recovered from treatment and demonstrated a normal progression to the blastocyst stage lead me to conclude that treatment with Rb1 or Rg1 alone does not replicate the outcomes observed following treatment with complete AQ or ALC extracts on preimplantation development.

Re is the second most prominent ginsenoside found in NAG extracts and is representative of the PT group. According to Chan et al. 2004, Re exerts a strong postimplantation embryotoxic effect starting with concentrations of 50 µg/ml. Re treatment severely disrupted development and was associated with a significant reduction in the morphological scores of rat post implantation embryo development (Chan et al. 2004). Contrastingly, when evaluating the effect of
ginsenoside Re on preimplantation development in vitro, no significant delays in embryonic development between the control and any treatment groups were found, even for high concentrations up to 89.6 µg/ml.

Finally, the influence of the PSF separated from the AQ ginseng extract on preimplantation development has not been investigated until now. In my present study, the effect of five different concentrations of PSF after 48 h in culture was determined. No significant inhibition of embryonic development was observed, even for concentrations up to 150 µg/ml. All treated embryos developed naturally and progressed to blastocyst stage once the extract was washed out and embryos were placed into fresh medium for 72 h. Thus the overall conclusion from all of the single agent treatment experiments is that while treatment with ginsenoside Rb1 and Rg1 using low concentrations certainly halted preimplantation development, neither these effects or the effect of treatment with Re or PSF negatively affected development overall. Therefore no single ginsenoside replicated the collective effects of AQ or ALC extract treatment observed on preimplantation development. Thus the impact on preimplantation development observed from AQ and ALC treatment must either result from an individual non-ginsenoside component not yet identified and experimented with or the collective effects of several of the ginseng extract components acting on the early embryo and impeding healthy embryonic development.

4.2 The Combinatorial Effect of Ginsenosides Rb1, Rg1 and Re on Mouse Preimplantation Development in Vitro
My subsequent experiments focused on attempting to define whether a combinatorial solution of ginsenosides could be responsible for the developmental blockade observed when preimplantation embryos were directly exposed to AQ and ALC ginseng extracts \textit{in vitro}. As previously shown, individual ginsenoside and PSF treatment did not replicate the effects observed when embryos were treated with whole extracts. Thus a combinatorial solution of Rb1, Rg1 and Re was used to treat the embryos for 48 h, in order to determine whether the developmental delay was due to the additive effects of a combination of ginsenosides. The effect of a combination of Rb1, Rg1 and Re on preimplantation development and reproduction has not been investigated until now. In my study, embryos were cultured with five different concentrations of the combinatorial solution for 48 h. No significant inhibition of embryonic development was observed for concentrations up to 257.1 µg/ml. Furthermore, all treated embryos developed naturally and progressed to the blastocyst stage once the extract was washed out and embryo was placed into fresh medium for 72 h. Thus the overall conclusion from this experiment is that treatment with a combination of ginsenosides Rb1, Rg1 and Re did not replicate the collective effects of AQ or ALC extract treatment observed on preimplantation development. Thus the impact on preimplantation development observed from AQ and ALC treatment must either result from a non-ginsenoside component not yet identified and experimented with or the collective effects of several of the ginseng extract compounds acting on the early embryo and impeding healthy embryonic development. Future experiments should be directed at determining the origin of the effects of ginseng extract treatment on preimplantation development.
development, even if oral consumption and in vivo studies do not indicate that ginseng consumption is detrimental to pregnancy and post-partum development.

4.4 Determine the Safety of Consuming both Ginseng Extracts on Pregnancy and Post-Partum Development in the Mouse

My results are the first to indicate that direct exposure to ginseng extracts is detrimental to preimplantation development. These outcomes are in sharp contrast to findings from in vivo studies investigating the influence of Red Korean Ginseng treatment two weeks before mating and throughout gestation on pregnancy in the mouse (Shin et al. 2010). It was determined that embryonic implantation and fetal growth were not negatively affected (Shin et al. 2010). Due to these discrepancies in outcomes between my culture study and Shin et al. 2010, it was essential to conduct in vivo experiments using NAG to determine the effects, if any, of these extracts on pregnancy and post-partum development in the mouse. Furthermore, since there are usually dose limitations in human studies, a lower dose of 50 mg/kg as well as an extremely high dose of 500 mg/kg (≈ 50 times the clinical recommended daily dose by KFDA) were investigated.

The first experiment consisted of treatment with 50 mg/kg of NAG extracts. Following parturition, the gestation period and litter size were determined as well as the sex ratio of mice weaned on post-partum day 20. No significant differences were observed between any treatments. Lastly, pup growth rate was determined by weighing individual pups every four days from birth to post-partum day 20 and no significant differences were observed between groups. In the second
experiment, females were gavaged with 500 mg/kg of ginseng extracts for two weeks prior to mating and throughout gestation. Interestingly, a significant difference of 0.68 days was observed between the sham and ALC group for gestation period. This difference of 0.68 days in mouse gestation would translate into nearly 9.5 days difference if a comparable effect were observed in humans. The average number of pups per litter between treatment groups also varied significantly between the control and gavaged treatments, implying that the gavage procedure certainly had some potential for affecting litter size. No significant differences in sex ratio were detected in any of the treatment groups. Additionally, pup growth rates from birth to post partum-day 20 were evaluated, and no developmental variations were observed in pup weight among treatment groups until post-partum day 20, where a significant difference between the ALC treatment and the three other groups (control, sham and AQ) was observed. To determine whether the observed difference in weight was due to possible variations in growth between males and females, weight was determined for each sex on day 20. While no differences were observed between treatment groups for female weight, a significant difference in male pup weight was observed on post-partum day 20 between the sham and ALC groups, indicating that pup growth rate differences may have been primarily due to variations in male weight, and not female growth. These results indicate that in vivo ALC ginseng treatment at very high dose of 500 mg/kg throughout gestation affect gestation period and also pup post-partum growth.

Thus, clear differences between administration of 50 and 500 mg/kg of both ginseng extracts were observed. It seems that while treatment with a low
dose does not affect pregnancy and post-partum development \textit{in vivo}, a high dose of 500 mg/kg of the ALC ginseng extract does increase gestation period and pup growth rates. The importance of these doses is uncertain. Most recommended levels for ginseng product consumption range from 1-2 g per day, indicating that a 100 kg person would have to ingest 50 g of ginseng daily to equal the 500 mg/kg level that I gavaged. Thus, even the 50 mg/kg/day is still high, however it is much closer to realistic consumption levels. In addition, my \textit{in vivo} results contrast dramatically with the effect of NAG on preimplantation embryos. This may be due to the fact that the preimplantation embryos were directly exposed to the ginseng extract in culture, whereas concentrations of ginseng directly affecting embryos \textit{in vivo} are unknown due to uncertainties regarding ginseng compound absorption, metabolism and renal clearance rate after consumption in the human. It is very likely that oral consumption results in very little if any embryonic exposure to ginseng compounds in the reproductive tract. The levels that could accumulate in the reproductive tract for any species are simply not known.

Studies have determined however, that maximum concentrations of Rb1, Rg1 and Re in rat plasma after an oral administration of 10 and 300mg/kg of ginseng powder were between 1.51 to 6.42 ug/ml (Li et al. 2007a). However, when Shin et al. 2010 attempted to measure Rb1 and Rg1 plasma concentrations in pregnant mice, no trace of these ginsenosides were found following oral treatment with an extremely high dose of 2000mg/kg/day of Red Korean ginseng after 30 min and 2h. Whether these results are transferable to humans, and intact ginsenosides are absorbed by the gastrointestinal tract and reach the systemic
circulation before first being metabolized is unknown. These results indicate that ginsenoside pharmacokinetics is poorly understood in general. The pharmacokinetics of ginseng extracts and placental transfer of ginsenosides in humans must be investigated further, but short half-life, poor absorption and rapid clearance may all contribute to very low exposure levels in the reproductive tract and could provide reasonable understanding as to why ginseng treatments differ so much with regards to effects to early development when comparing *in vitro* and *in vivo* treatment regimes.

Overall the results support a cautionary approach to determining whether ginseng extract consumption has a negative influence on pregnancy and post-partum development. Treatment with 50 mg/kg of NAG ginseng extract does not negatively affect pregnancy and post-partum development in the mouse. However, 500 mg/kg/day, certainly has an influence on litter size, and independent treatment with the ALC extract increases gestation period and pup growth when compared to the sham group. While my results with high levels of NAG do not agree with the outcomes from experiments using Korean Red ginseng, both studies did not observe a negative impact from administration of low levels of ginseng on pregnancy and post-partum development. While different experimental designs could be attempted, such as gavaging females before pregnancy only or throughout gestation only, and more experimental replicates could be added in order to increase the overall power of the study, it can be cautiously concluded that it is unlikely that oral consumption of ginseng at typical daily levels would negatively affect pregnancy and post-partum development in the mouse.
4.5 Conclusions

In conclusion, my study has revealed the following regarding ginseng extract effects on preimplantation development in culture and pregnancy and post-partum development in the mouse.

1. Treatment with either AQ or ALC NAG extracts results in a concentration dependent blockade of preimplantation development *in vitro*.

2. Treatment with 1000 ug/ml ALC extract does not negatively affect preimplantation development *in vitro*.

3. Preimplantation embryos treated with ALC extract are more likely to recover from treatment than embryos treated with AQ extract however, overall recovery from treatment is low for almost all treatments.

4. Treatment with Rg1, Rb1, Re and PSF alone does not replicate the effect of treatment with full AQ or ALC extract on preimplantation development.

5. Oral administration of 50 mg/kg/day AQ or ALC extract does not affect gestation, litter size, sex ratio or post-partum growth in the mouse.

6. Oral administration of 500 mg/kg/day AQ or ALC extract does affect gestation period and pup growth rate.

7. It is likely that oral consumption of typical daily levels of ginseng will not adversely affect pregnancy and post-partum development, which is probably due to a very low ginseng accumulation in the reproductive tract and thus low embryonic exposure to ginseng extract *in vivo*.

8. Future experiments should be directed towards determining ginseng extract metabolism, clearance rate and reproductive tract levels.
9. The mechanisms underlying the effects of ginseng treatment on preimplantation development *in vitro* should be investigated.
References

Akao, T., M. Kanaoka et al. (1998) Appearance of compound K, a major metabolite of ginsenoside Rb$_1$ by intestinal bacteria, in rat plasma after oral administration—measurement of compound K by enzyme immunoassay. Biol Pharm Bull. 21: 245–249


Li, X., J. Sun et al. (2007a) Simultaneous determination of panax notoginsenoside R1, ginsenoside Rg1, Rd, Re and Rb1 in rat plasma by HPLC/ ESI/MS: platform for the pharmacokinetic evaluation of total panax notoginsenoside, a typical kind of multiple constituent traditional Chinese medicine. Biomedical Chromatography. 21: 735-46
Li, X., G. Wang et al. (2007b) Pharmacokinetic and absolute bioavailability study of total panax notoginsenoside, a typical multiple constituent traditional chinese medicine (TCM) in rats. Biol Pharm Bull. 30: 847-51


Lim, J. H., T. C. Wen et al. (1997) Protection of ischemic hippocampal neurons by ginsenoside Rb1, a main ingredient of ginseng root. Neurosci Res. 28: 191-200

Liu, P., Y. Xu et al. (2005a) Developmental toxicity research of ginsenoside Rb1 using a whole mouse embryo culture model. Birth Defects Research. 74: 207-209


Park, J. D., D. K. Rhee et al. (2005) Biological activities and chemistry of saponins from panax ginseng C.A. Meyer. Phytochemistry Reviews. 4: 159-175


Shi, Q., Q. Hao et al. (2005) Ginsenoside-Rd from panax notoginseng enhances astrocyte differentiation from neural stem cells. Life Sci. 76: 983-95


Curriculum Vitae

Danyka Belanger  
Children’s Health Research Institute  
Victoria Research Labs, A-5-148  
800 Commissioners Rd, E  
London Ontario N6C 2V5

FORMAL EDUCATION

Master of Science – Physiology  
The University of Western Ontario  
London, Ontario, Canada  
2010-2012

Bachelors Degree – Honours Health Sciences  
University of Ottawa  
Ottawa, Ontario, Canada  
2005-2009

HONOURS AND AWARDS

CIHR Training Program in Reproduction, Early Development, and the Impact of Health Scholarship  
Canadian Institutes for Health Research  
2010-2012

Western Graduate Research Scholarship (WGRS)  
The University of Western Ontario  
London, Ontario, Canada  
2010-2012

Dean’s Honour List  
University of Ottawa  
Ottawa, Ontario, Canada  
2005-2009

Entrance Scholarship  
University of Ottawa  
Ottawa, Ontario, Canada  
2005-2006

RELATED WORK EXPERIENCE
**ORAL PRESENTATIONS**


**POSTER PRESENTATIONS**


Training Program in Reproduction, Early Development, and the Impact of Health, Ottawa ON, CAN