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COLLEGE OF HEALTH SCIENCES
SCHOOL OF MEDICINE
DEPARTMENT OF ANATOMY**



**Project paper on risk of Congenital Limb Reduction Defects
Associated with in utero exposure to Oral Contraceptives**

The project paper is submitted to Addis Ababa University, College of Health Sciences, School of Medicine, Department of Anatomy in Partial Fulfillment for Degree of Master of Science in Anatomy

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Lists of Abbreviation

AER	Apical Ectodermal Ridge
BCPs	Birth Control Pills
BMD	Bone Mineral Density
CL	Confidence limits
CP	Copulatory Plug
COCs	Combined Oral Contraceptive Pills
CYP3A	Cytochrome P450 enzymes
DES	Diethylstilbestrol
DMPA	Depot medroxyprogesterone acetate
EE	Ethinylestradiol
FDA	Food and Drug Administration
FSH	Follicle-stimulating hormone
GnRH	Gonadotropin-releasing hormone
LH	Luteinizing hormone
LMP	Last menstrual period
NA	Norethindrone
OCs	Oral Contraceptives
OCPs	Oral Contraceptive Pills
OR	Odds ratios
POP	Progestin-only pills
RR	Risk ratios
US	United States
WHO	World Health Organization

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Abstract

Oral contraceptives are widely used and are generally safe and effective for many women. Oral contraceptives are known also as the Pill, POP, COCPs, OCs, BCs, BC tablets, or birth control pills. This medicine usually contains two types of hormones, estrogens and progestins and, when taken properly, prevents pregnancy.

The aim of this project is to review and present the teratogenic effects of Oral contraceptives on the limb development.

Allegations that inadvertent pregnancies occurring in users of contraception are associated with congenital anomalies are common. Fortunately, there is little to no scientific basis for such claims. Evaluating these claims requires consideration of the two general mechanisms responsible for human malformation: teratogenesis and mutagenesis.

Some study indicate that there is a possibility of a sevenfold (7X) increase in risk of limb reduction defects, Other authors concluded that, exposure to sex hormones during pregnancy doubled the risk for some specific diagnoses, including certain limb defects, but these increases were not statistically significant.

Inbred normal adult SWR mice were used to investigate the possible teratogenic effect of Microginon 30 (0.15mg leronogestrel (L) + 0.03mg EE), as an oral contraceptive on fetuses of females receiving doses from day 7 to day 12 of pregnancy. External malformations including abnormal hind limb, abnormal tail and exencephaly have been induced in low frequencies by the doses 0.48L + 0.96E and 1.20L +0.24E mg/kg.

An analysis of available epidemiologic data leads the present reviewer to conclude that the use of exogenous hormones during human pregnancy has not been proved to cause developmental abnormality in non-genital organs and tissues. If there are increased risks of non-genital malformations associated with the administration of certain sex steroids, the risks are very small, may not be causal, and are substantially below the spontaneous risk of malformations.

Key words: Oral contraceptive, congenital limb reduction defects, pregnancy.

1. Introduction

1.1 Overview on Oral Contraceptives

Oral contraceptive pills are widely used and are generally safe and effective for many women. Oral contraceptives are known also as the Pill, Lo Loestrin Fe, Loette, Oral contraceptives (OCs), Progestin-only pills (POP), Birth Control (BCs), Birth Control tablets, or birth control pills (Abma and Chandra, 1997; Sylvia, 1999; Taylor, 2006).

Oral contraceptive pills (OCPs) contain two synthetic steroid hormones, namely ethinyl estradiol (EE) and progestins. Individual oral contraceptive packets differ in terms of their estrogen dosage, progestin type and dosage, cycle length, and hormone free interval (Bajos et al, 2007; Krishnan and Kiley, 2010). Ethinyl estradiol (EE) dosages have decreased with first generation OCPs containing 50 µg EE, second generation 20 µg to 35 µg EE and third generation 25 µg to 35 µg EE. Older progestins are derived from 19-nortestosterone (i.e., norethindrone, norethindrone acetate, ethynodiol diacetate, norethynodrel, levonorgestrel) while newer progestins (i.e., norgestimate, desogestrel, drospirenone) have less androgenic properties (Moreau and Gilbert, 2007).

Oral contraception is one of the keystones of reproductive health. Without contraception the individual is unable to choose when and how many children he/she will have. At a global level, contraception is important in helping reduce overcrowding, pressures on resources, pollution and global warming, and a loss of animal species due to loss of habitat. It is just as important at an individual level providing the means for individual couples to have the number of children they wish when they wish them. The health benefits of avoiding unplanned pregnancy and the ability to extend interbirth intervals have been well documented (Burkman et al., 2001 and Petitti, 2003).

1.1.1 Non-contraceptive health benefits of Oral contraception

There is an increasing awareness of the opportunity that many contraceptive interventions may provide for additional health benefits. Thus, the combined oral contraceptive pill in the short

term reduces many of the troublesome side effects associated with menstruation. In the long term, the risk of cancer of the ovary, uterus and probably also of the colon and rectum is reduced (Burkman et al., 2004). Long-term follow-up studies have found no evidence of increased or decreased mortality in women who have taken a combined oral contraceptive (Beral et al., 1999; Vessey et al., 2003). Societal benefits also increased from use of OCs, such as reduced disability due to dysmenorrhea and menorrhagia (Baird, 1965, 2000).

In a non-comparative trial, Larsson et al. (1992) reported that treatment with a low-dose OC significantly reduced dysmenorrhea. Fourteen of the 20 young women included in the trial complained of dysmenorrhea on inclusion before starting with a low-dose OC. After 6 months, treatment with a low-dose OC only 4 women reported dysmenorrhea. Progestogens and estrogen–progestogens are effective in the control of pain symptoms in approximately three in four women affected by endometriosis. Their effect does not seem to be inferior to that of other drugs used for the disease (Vercellini et al., 1993; Moore et al., 2003; Prentice et al., 2003). Acne is the most common skin condition treated by physicians, affecting up to 40% of adolescents and 10% of adult women which may improve during OC use as a noncontraceptive benefit (Redmond et al., 1997; Thiboutot et al., 2001; Haider and Shaw, 2004). For women with stress-related conditions (hypogonadotrophic or normogonadotrophic amenorrhoea), there is quite good evidence that OC use maintains or improves bone mineral density (BMD) (Castelo-Branco et al., 2001). OC use is associated with a reduced risk of fibroids (Ross *et al.*, 1986; Parazzini et al., 1988). Just as use of the combined pill is associated with a reduced risk of ovarian cancer, it has been suggested that the risk of benign ovarian tumours is also reduced (Holt et al., 1992). A number of studies have shown a reduced risk of functional ovarian cysts which is unsurprising since ovulation is inhibited (Holt et al., 1992). Oral contraceptive use may minimize the patient's ovarian & endometrial cancer (Schlesselman, 1999 and Lancet, 2008). These non-contraceptive benefits are not only an added advantage to women who use the method but also create an important public health benefit. Data from the Oxford-FPA study (Vessey et al., 1996) demonstrated that hospital referral of women for excessive, painful or irregular periods was reduced by 25–50% among women using the combined pill compared with nonusers.

1.1.2 Oral Contraceptives Formulations/ generation

The formulations of oral contraceptive pills have changed dramatically over the years. The first oral contraceptive pill, introduced in 1960, contained high doses of norethynodrel (progestin) and mestranol (estrogen) (Darney, 1995). Norethynodrel is one of the first-generation progestins called “estranes.” This class includes the current agents’ norethindrone, norethindrone acetate and ethynodiol diacetate. Levonorgestrel, a more potent, second-generation progestin, was developed in about 1970 (Kaplan, 1995). Over the past several decades, the dose of the estrogen component of oral contraceptive pills has decreased from the original 150 µg to 50 µg and then to 20 µg. These changes were made to lower the risk of thromboembolic complications associated with the use of oral contraceptive pills.

Originally, most combination oral contraceptive pill formulations were monophasic, with each active tablet containing a fixed dose of estrogen and progestin throughout the cycle. Multiphasic preparations (biphasic and triphasic) were developed in the 1980s to reduce the total dosage of progestin throughout the cycle without increasing the risk of break through bleeding (Chi, 1995 and Speroff, 1999).

Third-generation progestins from the gonane class were incorporated into oral contraceptive pill formulations to reduce the androgenic and metabolic side effects that occur with older agents. These new progestins include desogestrel, gestodene and norgestimate (Glass and Kase, 1999).

1.1.3 Oral Contraceptives structure

Levonorgestrel, which is a progestogen is a white, odourless crystalline powder. It is practically insoluble in water, slightly soluble in alcohol, acetone, ether, and soluble in chloroform. Chemically, levonorgestrel is (-)-13β-ethyl-17β-hydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3 and has the chemical structure as shown in Figure 1 below (Chilcott, 2012):

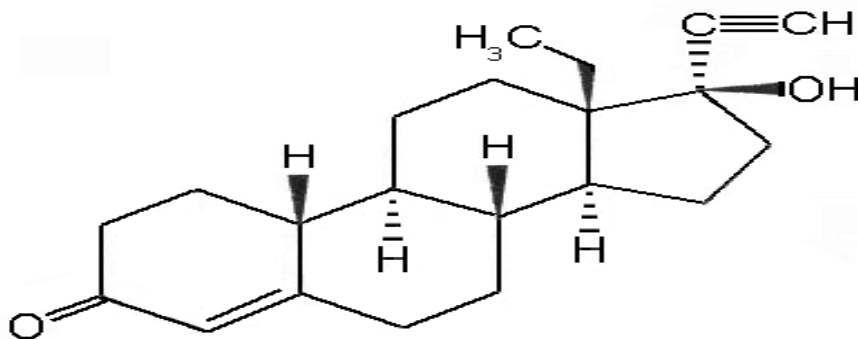


Fig. 1 chemical structure of Levonorgestrel (Chilcott, 2012)

Chemical Formula: C₂₁H₂₈O₂

Molecular Weight: 312.45

Melting Point: 232-239

CAS No: 797-63-7

Ethinylloestradiol, which is an oestrogen is a white to creamy white, odourless, crystalline powder. It is insoluble in water and soluble in alcohol, chloroform, ether, vegetable oils, and aqueous solutions of alkali hydroxides. Chemically, ethinylloestradiol is 19-nor-17 α -pregna-1,3,5(10)-trien-20-yne-3,17-diol and has the chemical structure as shown in Figure 1 below (Chilcott, 2012):

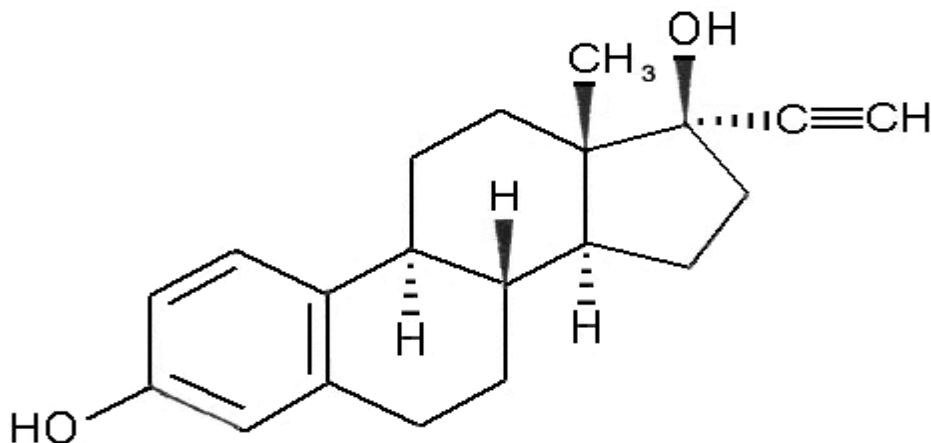


Fig. 2 chemical structure of ethinylloestradiol (Chilcott, 2012)

Chemical Formula: C₂₀H₂₄O₂

Molecular Weight: 296.41

Melting Point: 181-185°C

CAS No: 57-63-6

1.1.4 History of Oral Contraceptives

By the 1930s, scientists had isolated and determined the structure of the steroid hormones and found that high doses of androgens, estrogens or progesterone inhibited ovulation, (Goldzieher, 1974, 1982, 1993 and Perone, 1993). However, obtaining them from European pharmaceutical companies produced from animal extracts was extraordinarily expensive (Maisel, 1965).

In 1939, Russell Marker, a professor of organic chemistry at Pennsylvania State University, developed a method of synthesizing progesterone from plant steroid sapogenins, initially using sarsapogenin from sarsaparilla, which proved too expensive. After three years of extensive botanical research, he discovered a much better starting material, the saponin from inedible Mexican yams (*Dioscorea mexicana* and *Dioscorea composita*) found in the rain forests of Veracruz near Orizaba. The saponin could be converted in the lab to its aglycone moiety diosgenin. Unable to interest his research sponsor Parke-Davis in the commercial potential of synthesizing progesterone from Mexican yams, Marker left Penn State and in 1944 co-founded Syntex with two partners in Mexico City. When he left Syntex a year later the trade of the barbasco yam had started and the period of the heyday of the Mexican steroid industry had been started. Syntex broke the monopoly of European pharmaceutical companies on steroid hormones, reducing the price of progesterone almost 200-fold over the next eight years (Asbell, 1995; Lehmann, 1973; Marks, 2001; Watkins, 1998; Djerassi, 2001; Speroff, 2005 and Gereffi, 1983).

Midway through 20th century, the stage was set for the development of a hormonal contraceptive, but pharmaceutical companies, universities and governments showed no interest in pursuing the research (Tone, 2001).

Norethynodrel (and norethindrone) were subsequently discovered to be contaminated with a small percentage of the estrogen mestranol (an intermediate in their synthesis), with the norethynodrel in Rock's 1954–5 study containing 4–7% mestranol (Rock, 1957). When further purifying norethynodrel to contain less than 1% mestranol led to breakthrough bleeding, it was

decided to intentionally incorporate 2.2% mestranol, a percentage that was not associated with breakthrough bleeding, in the first contraceptive trials in women in 1956. The norethynodrel and mestranol combination was given the proprietary name Enovid (Pincus, 1958).

The first contraceptive trial of Enovid led by Edris Rice-Wray began in April 1956 in Río Piedras, Puerto Rico (Junod, 2002; Ramírez, 1983; Edris, 1957; Marsh, 2008). A second contraceptive trial of Enovid (and norethindrone) led by Edward T. Tyler began in June 1956 in Los Angeles (Vaughan, 1970; Olson, 1959). On January 23, 1957, Searle held a symposium reviewing gynecologic and contraceptive research on Enovid through 1956 and concluded Enovid's estrogen content could be reduced by 33% to lower the incidence of estrogenic gastrointestinal side effects without significantly increasing the incidence of breakthrough bleeding (Winter, 1957).

1.1.5 Effectiveness of Oral Contraceptives

The effectiveness of COCPs, as most forms of contraception, can be assessed in two ways. Perfect use or method effectiveness rates only include people who take the pills consistently and correctly. Actual use or typical use effectiveness rates are of all COCP users, including those who take the pills incorrectly, inconsistently, or both. Rates are generally presented for the first year of use (Trussell, 2007). Most commonly the Pearl Index is used to calculate effectiveness rates, but some studies use decrement tables (Kippley, 1996).

The typical use pregnancy rate among COCP users varies depending on the population being studied, ranging from 2-8% per year. The perfect use pregnancy rate of COCPs is 0.3% per year (Trussell, 2007).

COCPs provide effective contraception from the very first pill if started within five days of the beginning of the menstrual cycle (within five days of the first day of menstruation). If started at any other time in the menstrual cycle, COCPs provide effective contraception only after 7 consecutive days use of active pills, so a backup method of contraception must be used until active pills have been taken for 7 consecutive days. COCPs should be taken at approximately the same time every day (Leon, 2005 and FFPRHC, 2007).

Contraceptive efficacy may be impaired by: 1) missing more than one active pill in a packet, 2) delay in starting the next packet of active pills (i.e., extending the pill-free, inactive or placebo pill period beyond 7 days), 3) intestinal mal-absorption of active pills due to vomiting or diarrhea, 4) drug interactions with active pills that decrease contraceptive estrogen or progestogen levels (Leon, 2005).

1.1.6 Mechanism of action of Oral Contraceptives

Combined oral contraceptive pills were developed to prevent ovulation by suppressing the release of gonadotropins. Combined hormonal contraceptives, including COCPs, inhibit follicular development and prevent ovulation as a primary mechanism of action (Schwartz, 2007 and Glasier, 2010).

Progestogen negative feedback decreases the pulse frequency of gonadotropin-releasing hormone (GnRH) release by the hypothalamus, which decreases the secretion of follicle-stimulating hormone (FSH) and greatly decreases the secretion of luteinizing hormone (LH) by the anterior pituitary. Decreased levels of FSH inhibit follicular development, preventing an increase in estradiol levels. Progestogen negative feedback and the lack of estrogen positive feedback on LH secretion prevent a mid-cycle LH surge. Inhibition of follicular development and the absence of a LH surge prevent ovulation (Sulak, 2004 and Levin, 2011).

Estrogen was originally included in oral contraceptives for better cycle control (to stabilize the endometrium and thereby reduce the incidence of breakthrough bleeding), but was also found to inhibit follicular development and help prevent ovulation. Estrogen negative feedback on the anterior pituitary greatly decreases the secretion of FSH, which inhibits follicular development and helps prevent ovulation (Motan, 2008 and Darney, 2011).

Another primary mechanism of action of all progestogen-containing contraceptives is inhibition of sperm penetration through the cervix into the upper genital tract (uterus and fallopian tubes) by decreasing the water content and increasing the viscosity of the cervical mucus (Nelson, 2011).

The estrogen and progestogen in COCPs have other effects on the reproductive system, but these have not been shown to contribute to their contraceptive efficacy (Nelson, 2011):

- Slowing tubal motility and ova transport which may interfere with fertilization.
- Endometrial atrophy and alteration of metalloproteinase content, which may impede sperm motility and viability, or theoretically inhibit implantation.
- Endometrial edema, which may affect implantation.

Insufficient evidence exists on whether changes in the endometrium could actually prevent implantation. The primary mechanisms of action are so effective that the possibility of fertilization during COCP use is very small. Since pregnancy occurs despite endometrial changes when the primary mechanisms of action fail, endometrial changes are unlikely to play a significant role, if any, in the observed effectiveness of COCPs (Nelson, 2011).

1.2 Pharmacokinetics of Oral Contraceptives

1. Absorption

No specific investigation of the absolute bioavailability of Pills in humans has been conducted. However, literature indicates that levonorgestrel is rapidly and completely absorbed after oral administration (bioavailability about 100%) and is not subject to first-pass metabolism. Ethinylloestradiol is rapidly and almost completely absorbed from the gastrointestinal tract but due to first-pass metabolism in gut mucosa and liver, the bioavailability of ethinylloestradiol is between 38% and 48% (Bracken, 1990).

2. Distribution

In a study done by Bracken (1990), After a single dose of Pills to 22 women under fasting conditions, maximum serum concentrations of levonorgestrel are 2.8 ± 0.9 ng/mL (mean \pm SD) at 1.6 ± 0.9 hours. At steady state, attained from day 19 onwards, maximum levonorgestrel concentrations of 6.0 ± 2.7 ng/mL are reached at 1.5 ± 0.5 hours after the daily dose. The minimum serum levels of levonorgestrel at steady state are 1.9 ± 1.0 ng/mL. Observed levonorgestrel concentrations increased from day 1 (single dose) to days 6 and 21 (multiple

doses) by 34% and 96%, respectively. Unbound levonorgestrel concentrations increased from day 1 to days 6 and 21 by 25% and 83%, respectively. The kinetics of total levonorgestrel are nonlinear due to an increase in binding of levonorgestrel to sex hormone binding globulin (SHBG), which is attributed to increased SHBG levels that are induced by the daily administration of ethinylloestradiol. Levonorgestrel in serum is primarily bound to SHBG. Following a single dose, maximum serum concentrations of ethinylloestradiol of 62 ± 21 pg/mL are reached at 1.5 ± 0.5 hours. At steady state, attained from at least day 6 onwards, maximum concentrations of ethinylloestradiol were 77 ± 30 pg/mL and were reached at 1.3 ± 0.7 hours after the daily dose. The minimum serum levels of ethinylloestradiol at steady state are 10.5 ± 5.1 pg/mL. Ethinylloestradiol concentrations accumulated by 19% from days 1 to 21. Ethinylloestradiol is about 97% bound to plasma albumin. Ethinylloestradiol does not bind to SHBG, but induces SHBG synthesis.

3. Metabolism

Levonorgestrel: The most important metabolic pathway occurs in the reduction of the Δ^4 -3-oxo group and hydroxylation at positions 2α , 1β , and 16β , followed by conjugation. Most of the metabolites that circulate in the blood are sulfates of 3α , 5β -tetrahydro-levonorgestrel, while excretion occurs predominantly in the form of glucuronides (Pfizer, 2010). Some of the parent levonorgestrel also circulates as 17β -sulfate. Metabolic clearance rates may differ among individuals by several-fold, and this may account in part for the wide variation observed in levonorgestrel concentrations among users.

Ethinylloestradiol: Cytochrome P450 enzymes (CYP3A) in the liver are responsible for the 2-hydroxylation that is the major oxidative reaction. The 2-hydroxy metabolite is further transformed by methylation and glucuronidation prior to urinary and faecal excretion. Levels of Cytochrome P450 (CYP3A) vary widely among individuals and can explain the variation in rates of ethinylloestradiol 2-hydroxylation. Ethinylloestradiol is excreted in the urine and faeces as glucuronide and sulfate conjugates, and undergoes entero-hepatic circulation (Bracken, 1990).

4. Excretion

The elimination half-life for levonorgestrel is approximately 36 ± 13 hours at steady state (Pfizer, 2010). Levonorgestrel and its metabolites are primarily excreted in the urine (40% to 68%) and about 16% to 48% are excreted in faeces. The elimination half-life of ethinylloestradiol is 18 ± 4.7 hours at steady state (Pfizer, 2010).

1.3 Dosage and Administration of Oral contraceptives

Package quantities are available as: One-month packs containing one blister tray or a three-month pack containing 3 blister trays. Each blister tray contains 21 pink active tablets and 7 white inactive tablets. Each pink active tablet contains 100 µg levonorgestrel and 20 µg ethinylloestradiol and the excipients: microcrystalline cellulose, lactose, polacrillin potassium, magnesium stearate, macrogol 1450, Wax E, hypromellose, titanium dioxide and the colouring agent Iron Oxide Red CI 77491. Each white inactive tablet contains microcrystalline cellulose, lactose, polacrillin potassium, magnesium stearate, macrogol 1450, Wax E, hypromellose, and titanium dioxide (Pfizer, 2010).

On the first day of the menstrual cycle, i.e. the first day of bleeding, the woman is instructed to take a pink active tablet corresponding to that day of the week from the pink shaded section of the pack. Thereafter, one pink active tablet is taken daily, following the arrows marked on the package, until all 21 pink active tablets have been taken. The woman is then instructed to take one white inactive tablet daily for the next seven days following the arrows marked on the pack. Withdrawal bleeding should usually occur within 2 to 4 days after the last pink active tablet is taken. It is effective from the first day of therapy if the tablets are begun on Day 1 as described. Starting on days 2-7 is allowed, but during the first cycle a back-up method of contraception is recommended for the first 7 days of tablet taking. The back-up method of contraception must be an additional non-hormonal barrier method such as condoms or a diaphragm with a spermicide. Back-up contraception does not include the rhythm or temperature methods. The next and all subsequent courses of pack will begin on the day after the last package was completed, even if withdrawal bleeding is still in progress. Each course of

pack is thus begun on the same day of the week as the first course. Any time a new cycle of pack is started later than the eighth day after discontinuance of the pink active tablet, the woman should use a back-up non-hormonal method of contraception (other than the rhythm or temperature methods), until an active pink tablet has been taken for 7 consecutive days (Bracken, 1990).

Management of Missed Tablets

Contraceptive efficacy may be reduced if tablets are missed and particularly if the missed tablets extend the inactive tablet interval. If tablets were missed in the first week of the cycle and intercourse took place in the week before the tablets were missed, the possibility of a pregnancy should be considered (Kenny et al., 2012):

- If one active pink tablet is missed, but is **less than 12 hours late**, it should be taken as soon as it is remembered. Subsequent tablets should be taken at the usual time.
- If one active pink tablet is missed and is **more than 12 hours late** or if more than one active tablet is missed, contraceptive protection may be reduced.

The last missed pink tablet should be taken as soon as it is remembered, even if this means taking two active pink tablets in one day. Any earlier missed tablets should be discarded. The woman should then continue to take tablets at her usual time. In addition, a non-hormonal back-up method of contraception (other than the rhythm or temperature methods) should be used until one active tablet has been taken daily for 7 consecutive days (Willacy et al., 2012):

- If the 7 days where back up is required run beyond the last active pink tablet in the current pack, the next pack must be started on the day following the intake of the last pink tablet in the current pack. All inactive (white) tablets should be discarded. This prevents an extended break in the active tablet taking that may increase the risk of escape ovulation. The woman is unlikely to have a withdrawal bleed until the inactive tablet interval of the second pack, but she may experience spotting or breakthrough bleeding on active tablet taking days.

- If the user does not have a withdrawal bleed at the end of the second pack, the possibility of pregnancy must be ruled out before resuming tablet taking.

If the woman misses one or more white inactive tablets, she will still be protected against pregnancy provided she begins the pink active tablets on the appropriate day (Kenny et al., 2012).

Table 1 A table showing what to do if once missed any pills from a 21-day or 28-day pack of combination pills (Kenny et al., 2012).

Number of Pills Missed	When Pills Missed	What to do ...	Seven-Day Backup Needed?
First 1–2 pills	Beginning of pack	<ul style="list-style-type: none"> • Woman takes a pill as soon as she remembers. • Take the next pill at the usual time. (This means she may take two pills in one day.) 	Yes
1–2 pills	Day 3–day 21	<ul style="list-style-type: none"> • Woman takes a pill as soon as she remembers. • Take the next pill at the usual time. (This means she may take two pills in one day.) 	No
3 or more pills	First two weeks	<ul style="list-style-type: none"> • Woman takes a pill as soon as she remembers. • Take the next pill at the usual time. (This means she may take two pills in one day.) 	Yes
3 or more pills	Third week	<ul style="list-style-type: none"> • Do not finish pack. Throw away remaining pills. • Start next pack. 	Yes
1–7 reminder pills	Fourth week	<ul style="list-style-type: none"> • Throw away the missed reminder pill(s). • Take next reminder pill at the usual time. 	No

If vomiting or diarrhoea occurs during or shortly after the intake of pack, contraceptive reliability may be jeopardised. If vomiting or diarrhoea occurs within 4 hours after tablet taking, absorption may not be complete. In such an event, the advice concerning Management of Missed Tablets is applicable. The woman must take the extra active tablet(s) needed from a back-up pack (Willacy et al., 2012).

1.4 Limbs Development

1.4.1 Early Stages of Limb Development

Limb development begins with the activation of a group of mesenchymal cells in the lateral mesoderm. Homeobox genes regulate patterning in the formation of the limbs. The limb buds form deep to a thick band of ectoderm (AER). Toward the end of the fourth week, the limb buds first appear as elevations of the ventrolateral body wall. The upper limb buds are visible by day 26 or 27, and the lower limb buds appear 1 or 2 days later. Each limb bud consists of a mass of mesenchyme covered by ectoderm. The mesenchyme is derived from the somatic layer of lateral mesoderm (Riddle, 1999).

The limb buds elongate by proliferation of the mesenchyme. The upper limb buds appear disproportionately low on the embryo's trunk because of the early development of the cranial half of the embryo. The earliest stages of limb development are alike for the upper and lower limbs. Because of their form and function, there are many distinct differences between the development of the hand and foot. The upper limb buds develop opposite the caudal cervical segments and the lower limb buds form opposite the lumbar and upper sacral segments.

At the apex of each limb bud, the ectoderm thickens to form an apical ectodermal ridge (AER). The AER, a multilayered epithelial structure, is induced by the underlying mesenchyme. Bone morphogenetic protein signaling is required for its formation. The AER exerts an inductive influence on the limb mesenchyme that initiates growth and development of the limbs in a proximo-distal axis. Experimental studies show that expression of endogenous fibroblast growth factors and T-box genes (tbx-4 and tbx-5) in the AER are essential for this process (Riddle, 1999). Mesenchymal cells aggregate at the posterior margin of the limb bud to form the zone of polarizing activity. Fibroblast growth factors from the AER activate the zone of

polarizing activity, which causes expression of the sonic hedgehog (Shh) genes. It has been suggested that Shh secretions (morphogens) control the patterning of the limb along the anterior-posterior axis (Martin, 1998).

Expression of Wnt7 from the dorsal epidermis of the limb bud and engrailed-1 (EN-1) from the ventral aspect are involved in specifying the dorsal-ventral axis. The AER itself is maintained by inductive signals from Shh and Wnt7. The mesenchyme adjacent to the AER consists of undifferentiated, rapidly proliferating cells, whereas mesenchymal cells proximal to it differentiate into blood vessels and cartilage bone models. The distal ends of the limb buds flatten into paddle-like hand- and footplates. Laboratory studies have shown that endogenous retinoic acid is also involved in limb development and pattern formation.

By the end of the sixth week, mesenchymal tissue in the handplates has condensed to form digital rays. These mesenchymal condensations outline the pattern of the digits or fingers. During the seventh week, similar condensations of mesenchyme form digital rays and toes in the footplates. At the tip of each digital ray, a part of the AER induces development of the mesenchyme into the mesenchymal primordia of the bones (phalanges) in the digits. The intervals between the digital rays are occupied by loose mesenchyme. Soon the intervening regions of mesenchyme break down, forming notches between the digital rays. As the tissue breakdown progresses, separate digits (fingers and toes) are formed by the end of the eighth week. Programmed cell death (apoptosis) is responsible for the tissue breakdown in the interdigital regions, and it is probably mediated by bone morphogenetic proteins, signaling molecules of the transforming growth factor superfamily. Blocking these cellular and molecular events could account for syndactyly or webbing of the fingers or toes (Martin, 1998).

1.4.2 Final Stages of Limb Development

As the limbs elongate, mesenchymal models of the bones are formed by cellular aggregations. Chondrification centers appear in the fifth week. By the end of the sixth week, the entire limb skeleton is cartilaginous.

Osteogenesis of long bones begins in the seventh week from primary ossification centers in the middle of the cartilaginous models of the long bones. Ossification centers are present in all long bones by the 12th week. Ossification of the carpal (wrist) bones only begins during the first year

after birth (Slack, 2006).

From the dermomyotome regions of the somites, myogenic precursor cells also migrate into the limb buds and later differentiate into myoblasts, precursors of muscle cells. As the long bones form, the myoblasts aggregate and form a large muscle mass in each limb bud. In general, this muscle mass separates into dorsal (extensor) and ventral (flexor) components. The mesenchyme in the limb bud also gives rise to ligaments and blood vessels. The cervical and lumbosacral myotomes contribute to the muscles of the pectoral and pelvic girdles, respectively.

Early in the seventh week, the limbs extend ventrally. Originally the flexor aspect of the limbs is ventral and the extensor aspect dorsal and the preaxial and postaxial borders are cranial and caudal, respectively. The developing upper and lower limbs rotate in opposite directions and to different degrees (Zuniga, 2005):

- The upper limbs rotate laterally through 90 degrees on their longitudinal axes; thus, the future elbows come to point dorsally and the extensor muscles lie on the lateral and posterior aspects of the limb.
- The lower limbs rotate medially through almost 90 degrees; thus, the future knees come to face ventrally and the extensor muscles lie on the anterior aspect of the lower limb.

Developmentally, the radius and the tibia are homologous bones, as are the ulna and fibula, just as the thumb and great toe are homologous digits. Synovial joints appear at the beginning of the fetal period, coinciding with functional differentiation of the limb muscles and their innervation.

Cutaneous Innervation of Limbs

There is a strong relationship between the growth and rotation of the limbs and this cutaneous segmental nerve supply. Motor axons arising from the spinal cord enter the limb buds during the fifth week and grow into the dorsal and ventral muscle masses. Sensory axons enter the limb buds after the motor axons and use them for guidance. Neural crest cells, the precursors of Schwann cells, surround the motor and sensory nerve fibers in the limbs and form the neurilemmal and myelin sheaths (Slack 2006).

During the fifth week, peripheral nerves grow from the developing limb plexuses (brachial and lumbosacral) into the mesenchyme of the limb. The spinal nerves are distributed in segmental bands, supplying both dorsal and ventral surfaces of the limb. A dermatome is the area of skin supplied by a single spinal nerve and its spinal ganglion; however, cutaneous nerve areas and dermatomes show considerable overlapping. As the limbs elongate, the cutaneous distribution of the spinal nerves migrates along the limbs and no longer reaches the surface in the distal part of the limbs. Although the original dermatomal pattern changes during growth of the limbs, an orderly sequence of distribution can still be recognized in the adult (Moore, 2006).

1.4.3 Congenital Anatomic Anomalies or Human Birth Defects

Congenital anatomic anomalies, birth defects, and congenital malformations are terms currently used to describe developmental disorders present at birth. Birth defects are the leading cause of infant mortality and may be structural, functional, metabolic, behavioral, or hereditary (Hudgins and Cassidy, 2006).

A. Classification of Birth Defects

The most widely used reference guide for classifying birth defects is the International Classification of Diseases (Medicodes' Hospital and Payer, 1995); however, no single classification has universal appeal. Each is limited, having been designed for a particular purpose. Attempts to classify congenital anatomic anomalies or human birth defects, especially those that result from errors of morphogenesis, reveal the frustration and obvious difficulties in the formulation of concrete proposals that could be used in medical practice (Hudgins, 2006). A practical classification system for congenital anomalies that takes into consideration the time at onset of the injury, possible etiology, and pathogenesis is now widely accepted among clinicians.

B. Causes of congenital anatomic anomalies

The causes of congenital anatomic anomalies or birth defects are often divided into:

- Genetic factors such as chromosome abnormalities
- Environmental factors such as drugs and viruses
- Multi-factorial inheritance (genetic and environmental factors acting together in a

complex manner).

Note that the causes of most anomalies are unknown and that 20% to 25% of them are caused by a combination of genetic and environmental factors (multi-factorial inheritance) (Paget, 1882).

For 50% to 60% of congenital anomalies, the etiology is unknown (Lancet, 1882). The anomalies may be single or multiple and of major or minor clinical significance. Single minor anomalies are present in approximately 14% of newborns (Paget, 1882). Anomalies of the external ear, for example, are of no serious medical significance, but they indicate the possible presence of associated major anomalies. For example, the presence of a single umbilical artery alerts the clinician to the possible presence of cardiovascular and renal anomalies. Ninety percent of infants with three or more minor anomalies also have one or more major defects (Lancet, 1882). Of the 3% born with clinically significant congenital anomalies, 0.7% has multiple major defects (Lancet, 1882). Most of these infants die during infancy. Major developmental defects are much more common in early embryos (10%-15%); however, most of them abort spontaneously during the first 6 weeks. Chromosome abnormalities are present in 50% to 60% of spontaneously aborted embryos (Paget and Lancet, 1882).

1.4.4 Anomalies of the Limbs

Minor limb anomalies are relatively common and can usually be corrected surgically. Although these anomalies are usually of no serious medical consequence, they may serve as indicators of more serious anomalies and may be part of a recognizable pattern of birth defects.

The most critical period of limb development is from 24 to 36 days after fertilization. This statement is based on clinical studies of infants exposed to thalidomide, a potent human teratogen, during the embryonic period (Cooperman et al., 2006). Exposure to this teratogen before day 33 may cause severe limb defects, such as amelia, the absence of limbs. Consequently, a teratogen that could cause amelia of the limbs or parts of them must act before 36 days, the end of the critical period of limb development. Many severe limb anomalies occurred from 1957 to 1962 as a result of maternal ingestion of thalidomide. This drug, widely

used as a sedative and antinauseant, was withdrawn from the market in December 1961. Since that time, similar limb anomalies have rarely been observed. Because thalidomide is now used for the treatment of leprosy and several other disorders, it must be emphasized that thalidomide is absolutely contraindicated in women of child-bearing age (Cooperman et al., 2006).

Major limb anomalies appear approximately twice in 1000 newborns (Roessler et al., 1996). Most of these defects are caused by genetic factors. Molecular studies have implicated gene mutation (Hox gene, BMP, Shh, Wnt7, En-1, and others) in some cases of limb defects. Several unrelated congenital anomalies of the lower limb were found to be associated with a similar aberrant arterial pattern, which might be of some importance in the pathogenesis of these defects (Roessler et al., 1996).

Causes of Limb Anomalies

Anomalies or defects of the limbs originate at different stages of development. Suppression of limb bud development during the early part of the fourth week results in absence of the limbs, amelia. Arrest or disturbance of differentiation or growth of the limbs during the fifth week results in various types of meromelia. As summarized by Paget and Lancet (1882), limb defects are caused by:

- Genetic factors, e.g., chromosomal abnormalities associated with trisomy 18
- Mutant genes as in brachydactyly or osteogenesis imperfecta, a severe limb defect with fractures occurring before birth
- Environmental factors, e.g., teratogens such as thalidomide
- A combination of genetic and environmental factors (*multifactorial inheritance*), e.g., congenital dislocation of the hip
- Vascular disruption and ischemia, e.g., limb reduction defects

Experimental studies support the suggestion that mechanical influences during intrauterine development may cause some limb defects. A reduced quantity of amniotic fluid (*oligohydramnios*) is commonly associated with limb deformations (Van, 1996).

The most commonly occurring congenital anomalies of Limb are as listed below (Van, 1996):

Cleft Hand and Cleft Foot

In **lobster-claw deformities**, there is absence of one or more central digits, resulting from failure of development of one or more digital rays. The hand or foot is divided into two parts that oppose each other like lobster claws.

Congenital Absence of the Radius

The radius is partially or completely absent. The hand deviates laterally (radially), and the ulna bows with the concavity on the lateral side of the forearm. This anomaly results from failure of the mesenchymal primordium of the radius to form during the fifth week of development. Absence of the radius is usually caused by genetic factors.

Brachydactyly

Shortness of the digits (fingers or toes) is the result of reduction in the length of the phalanges. This anomaly is usually inherited as a dominant trait and is often associated with shortness of stature.

Polydactyly

The term supernumerary digits refer to the presence of more than the usual number of fingers or toes. Often the extra digit is incompletely formed and lacks normal muscular development. If the hand is affected, the extra digit is most commonly medial or lateral rather than central. In the foot, the extra toe is usually on the lateral side. Polydactyly is inherited as a dominant trait.

Syndactyly

Syndactyly is the most common anomaly of the hand or foot. Cutaneous syndactyly (simple webbing between digits) is the most common limb anomaly. It is more frequent in the foot than in the hand. Cutaneous syndactyly results from failure of the webs to degenerate between two or more digits. Osseous syndactyly (fusion of the bones-*synostosis*) occurs when the notches between the digital rays fail to develop; as a result,

separation of the digits does not occur. Syndactyly is most frequently observed between the third and fourth fingers and between the second and third toes. It is inherited as a simple dominant or simple recessive trait. A case of synpolydactyly (syndactyly and polydactyly), caused by mutations in the NH₂-terminal, non-DNA binding part of *HoxD13*, has been reported (Van, 1996).

Congenital Clubfoot

Any deformity of the foot involving the talus (ankle bone) is called talipes or clubfoot. Talipes equinovarus is the most common type. Clubfoot is a relatively common anomaly, occurring approximately once in 1000 births. It is characterized by an abnormal position of the foot that prevents normal weight bearing. The sole of the foot is turned medially and the foot is inverted. Clubfoot is bilateral in approximately 50% of cases, and it occurs approximately twice as frequently in males. The cause of clubfoot is uncertain. Although it is commonly stated that clubfoot results from abnormal positioning or restricted movement of the fetus's lower limbs in utero, the evidence of this deformation is inconclusive (Van, 1996). Clubfoot appears to follow a multifactorial pattern of inheritance; hence, any intrauterine position that results in abnormal positioning of the feet may cause clubfeet if the fetus is genetically predisposed to this deformity.

Congenital Dislocation of the Hip

This deformity occurs approximately once in 1500 newborn infants and is more common in females than in males. The joint capsule is very relaxed at birth, and there is underdevelopment of the acetabulum of the hip bone and the head of femur. Dislocation almost always occurs after birth. There are two causative factors (Van, 1996):

- Abnormal development of the acetabulum occurs in approximately 15% of infants with congenital dislocation of the hip, which is common after breech deliveries, suggesting that breech posture during the terminal months of pregnancy may result in abnormal development of the acetabulum and the head of femur.
- Generalized joint laxity is often a dominantly inherited condition that appears to be associated with congenital dislocation of the hip, which follows a multifactorial pattern of inheritance.

2. Objective

2.1 General Objective

- To review and present the teratogenic effects of Oral Contraceptives on the limb development.

2.2 Specific objective

- To review and present the role of oral contraceptive on limb development.
- To review and present the effects of oral contraceptive on limb development.
- To review and present the different factors that determines the teratogenicity of oral contraceptive on the limb development.
- To review and present the role of oral contraceptive on non-genital tissues.

3. Methodology

The methodology employed to prepare this project will be on the subject matter and analyses the different articles to come up to a conclusion. The articles will be gathered by reviewing different published journal articles and books, primarily by browsing different journal catalogs on the internet like PubMed, Medline, Hinnary and Google Scholar to identify relevant articles that have been published.

4. Findings and Discussions of the review of published researches on the teratogenic effects of Oral Contraceptives in the development of the limbs.

4.1 Oral Contraceptives and Teratogenicity

There is much information on OCs use during pregnancy since it is taken for the prevention of pregnancy, and will not interrupt an existing pregnancy. A review of the literature on inadvertent use of oral contraceptives (OCs) during pregnancy provides the most information relevant to fetal exposure to sex hormones. There are many reported cases of women inadvertently taking OCs, as either combination hormonal pills containing an estrogen and a progestin, or progestin-only pills (POPs), for up to several months while pregnant. The comprehensive reviews analyzed here provide strong evidence that exposure to sex hormones [both combination hormonal products and levonorgestrel-alone pills] in early pregnancy does not have a teratogenic effect. Much of the epidemiologic literature dates to the 70s and 80s when use of higher-dose oral contraceptives than currently prescribed was extensive, and reports of congenital anomalies were being analyzed as to general risk factors and maternal medications around the time of conception or during pregnancy.

The following are important review articles about teratogenic risk with sex hormone exposure around the time of conception and during the first trimester of pregnancy. These articles summarize the current state of science and primarily rely on clinical trials and prospective, cohort studies, which have less bias than case-control observational studies.

Limb reduction defects are defined as shortening or absence of a limb, finger, or a toe. Although genetic heterogeneity exists, most limb reduction defects can be considered polygenic/multifactorial in etiology. Janerich et al. (1974) were the first to report an association between progestogens and limb reduction defects. Of 108 women with an affected infant, 15 had received sex hormones (inadvertent oral contraceptive exposure, hormone pregnancy test, or hormones for pregnancy maintenance). Only four of 108 controls were exposed to sex hormones ($p < .05$). The association was not significant when oral contraceptives were considered separately.

This detailed review of the literature examined 18 major prospective studies evaluating the effects of progestin exposure during pregnancy, and determined that the doses received were not teratogenic (Simpson and Phillips, 1990). Overall, the authors analyzed data from 6,102 exposed and 83,167 unexposed women. They point out that it is improbable that a single source of bias would have influenced all the studies systematically. They concluded that the overall relative risk for all malformations from the 12 prospective cohort studies was the same for exposed and unexposed women. "This lack of association between OCs and birth defects in prospective studies agrees with the results of most of the better-designed case-control studies" (Oakley et al., 1973 and Bracken, 1990).

A well-constructed case-control study failed to observe a relationship in which the control group consisted of women who had delivered children with chromosomal abnormalities. Use of this abnormal control group obviated potential for recall bias (Oakley et al., 1973). Among other case-control studies is that of Lammer and Cordero (1986), among 1091 infants with major malformations, no significant association was found between hormone exposure and limb reduction defects. The study of Pardthaisong et al. (1988) also failed to demonstrate an association between limb reduction defects and use of oral contraceptives, either prior to conception or during gestation.

A retrospective case-control study in Australia observed an association between oral contraceptive use and limb abnormalities. Of 155 limb-deficient children, 18 were reported as having been exposed, compared with one normal control (RR, 16.6; $p < .05$). No association was found when progestin use only was considered. The anomalies were varied, including dysmelic longitudinal defects (e.g., absence of radius or thumb) and transverse defects (e.g., amputation of limbs or digits). Unfortunately, serious methodological shortcomings exist with this study. The potential for recall and memory bias was enormous. Interviews were conducted on the average 4.5 years after the birth of the child! Moreover, the crucial issue of when during gestation the fetus was exposed was not addressed (Krickler et al., 1986).

Cohort studies have further failed to confirm an association, as shown in Table 2. Prospective studies investigating limb reduction defects have specific validity because missing digits or severe limb shortening should be obvious to even the casual observer, of most interest is the

study of Yovich et al., (1988), who found no limb reduction defects among 508 exposed pregnancies. Also of interest is the failure of Nishimura et al., (1974) to observe limb reduction deformities in 108 micro-dissected embryos recovered from progestin-exposed mothers.

In a case-control study of 8816 newborns in Thailand, Pardthaisong et al.,(1988) obtained a contraceptive history on the mothers finding 1229 women who used injectable contraceptive Depot medroxyprogesterone acetate (DMPA), 3038 having used oral contraceptives before conception or during pregnancy and 4549 that used no contraceptive use before conception. The frequency of peripheral limb abnormalities, polydactyly and syndactyly was significantly increased in DMPA users (RR, 4.9/1000) compared to users of oral contraceptives (RR, 1.2/1000); no significant difference was seen in comparison to women receiving no contraception (1.7/1000). However, only three cases of polysyndactyly were exposed during the critical period of limb organogenesis (days 28 to 52). The rates of polysyndactyly observed in DMPA users were higher than those reported in other studies, for which reason, the authors concluded that the observed association may be the result of genetic predisposition in this population. They concur, noting as well that neither other case-control study nor cohort study (see Table 1) reported an association between polydactyly and progestogens.

Table 2 Summary on Major Prospective Studies Conducted to Evaluate Effects of Progestin Exposure during Pregnancy and their Findings* (Modified from Simpson JL and Phillips OP, 2008).

Investigator	Sample	Control	Anomalies	Comments
Spira et al (1972)	9566 women, interviewed in the 3rd month, who received hormones (mostly for pregnancy support or diagnosis) (France)		171/9566 (1.8%)	Anomalies were found with equal frequency in exposed and unexposed pregnancies.
		8387 not receiving hormones	168/8387 (2%)	
Harlap et al (1975)	11,468 women, 432 receiving "hormones" (Israel)		47/432 (10.9%) all anomalies, 21/432 (4.9%) major anomalies only	Small increase (25%) (p < .02) observed, but recall bias possible because interviews were months after exposure
		11,036 unexposed	925/11,036 (8.4%): 426/11,036 (3.9%), major only	
Kullander and Kallen (1976)	6379 pregnancies in which 194 mothers had abnormal infants (Sweden)		5/194 exposed to progestogen (2.6%)	Exposure rates similar in both groups
		5002 women delivered normal infants	98/5002 exposed to progestogen (2.0%)	
Royal College of General Practitioners	136 pregnancies conceived during oral contraceptive therapy		2/136 (1.5%)	No differences
		11,009 pregnancies in	177/11,009 (1.6%)	

Investigator	Sample	Control	Anomalies	Comments
(1976)		nonusers;		between the groups
		5,530 pregnancies in previous contraceptive users	86/5530 (1.6%)	
Goujard and Rumeau-Rouquette (1977)	12,895 mothers interviewed in the first trimester, of whom 1165 were exposed (France) (same population as Spira et al;1972)		5/335 (1.5%) "testosterone derivatives"; 15/830 (1.8% "progesterone derivatives"	Chromosomal anomalies excluded from analysis; no differences observed either overall or after separate analysis for cardiac and skeletal defects
		9822 nonexposed	160/9822 (91.6%) nonexposed	
Goujard et al (1979)	3451 women, of whom 133 used progestins (France)		5/133 (3.8%)	Four of 5 anomalies occurring in subset of 35 women who used testosterone derivatives
		3318 nonexposed	3318 (2.3%) overall	
Savolainen et al (1981)	3002 mothers of malformed infants, of whom 38 conceived while receiving "pills" (Finland)			Anomaly rates similar in sample and control, both for previous or concurrent contraceptive use
		3002 matched controls		
	150 pregnancies treated with		1/150 (0.7%)	

Investigator	Sample	Control	Anomalies	Comments
Varma and Morsman (1982)	hydroxyprogesterone lexoneate from 6-18 weeks of gestation for repetitive abortions			No significant difference between exposed and unexposed subjects
		Matched controls of women with abortions	3/150 (2.0%)	
Michaelis et al (1983)	13,643 pregnancies, about 10% of whom received hormones for diagnosis or support		4/320 (1.3%), progesterone along; 11/610 (1.8%), progesterone and estradiol	No significant difference between exposed cases and their unexposed matched controls
		Matched controls within same population who were not exposed		

*The consensus is that progestins in the doses given were not teratogenic.

From the above cohort studies, (see Table 2) only Goujard et al., (1979) claimed an association between progestins and overall anomaly rate. One of the two Harlap et al., (1975) studies showed a small increase in RR. The other prospective studies failed to show any generalized increase in anomalies. Resseguie et al., (1985) and Katz et al., (1985) failed to detect an increase in the major anomaly rate of offspring exposed in utero to progestins. Yovich et al., (1988) also found no significant increase in congenital abnormalities in pregnancies exposed to medroxy-progesterone (15 of 366 or 4.1%) as compared to the controls (15 of 428 or 3.5%).

In a case control study, one hundred and seventy four pairs were admitted to the study, but 59(33.9%) were excluded; in 40 cases this was because the child's condition was dwarfism, arthrogryphosis or achondroplasia rather than a limb reduction defect. Of the remainder, 10 were excluded because of inability to trace the family, inability of the family practitioner to

cooperate in six cases, and no part time medical officer being available for three cases, leaving 115 for analysis. Odds ratios (OR) and 95% confidence limits (CL) were calculated using matched pair analysis (Breslow, 1980 and Rothman, 1982). They concluded that no significant association was found between Oral Contraceptives and limb reduction defects.

There were also authors who reviewed the birth certificates and hospital records of 7723 infants whose mothers had reported used oral contraceptives (Rothman and Louik, 1978). The overall frequency of malformation was 4.3% for infants whose mothers terminated use of oral contraceptives shortly before conception, as compared with 3.3% for infants whose mothers did not take oral contraceptives during the three years before conception. The 90 per cent confidence limits for the prevalence ratio were 1.0 and 1.7. No difference was apparent for major malformations. For specific malformations the most notable difference was for undescended testis, but this excess, like the overall excess, could be explained by sampling variability (Rothman and Louik, 1978).

Clinical trials of NORPLANT® system found no evidence of teratogenicity of levonorgestrel administered by implants (Population Council, 1990). The four major teratogenic concerns associated with sex hormones are heart, limb, vertebral, and GI tract anomalies. The authors concluded that there is no association between oral contraceptives and birth defects based on several findings, including extensive surveillance data that did not show a corresponding rise in the incidence of the suspected birth defects as the use of OCs increased. They pointed out that because sex hormones act specifically on tissues with hormone receptors that are primarily on reproductive/genital tissues, the probability of receptor binding causing anomalies on non-genital tissue is low (Wilson et al.,1981). However, exposure to levonorgestrel cause birth defects on genital tissues. For example it cause penile or perineoscrotal hypospadias, ureter, bladder, and urethral anomalies and undescended testis (Wilson et al., 1981).

In a case-control study, seven of 76 mothers that delivered infants with transposition of the great vessels had received hormones in the first trimester; none (0) of the 76 controls had been exposed to sex hormones ($p < 0.007$) (Levy et al.,1973). Similar findings were published by Nora and Nora, (1973) whose initial retrospective case-control study found that 20 of 224 (8.9%) mothers delivered infants with cardiac defects recollected receiving an estrogen/progesterone

compound; 4 of 262 controls ($p < 0.001$) were similarly affected. However, in a follow-up prospective study, Nora et al., (1978) reported no significant differences in hormone use between the 60 mothers and their controls. Despite these negative findings, a second prospective study was undertaken with two controls per subject. In this second study, 31 of 176 mothers with affected offsprings were said to have received sex hormones, compared with 21 of 352 control mothers ($p < 0.001$).

Two other early case-control studies reported positive correlations. Janerich et al., (1977) identified 104 infants with cardiac defects through birth certificates in New York State. Of the 104, mothers of 18 received sex hormones; 16 cases involved hormones for pregnancy diagnosis and two involved inadvertent contraceptive use. Significantly fewer controls reported exposure.

Although troublesome, these studies were counterbalanced by other negative case-control studies. In a case-control study of 1370 children with congenital malformations and exposure to oral contraceptives “around the time of conception,” Bracken et al., (1978) found no significant increase in the rates of certain cardiac malformations (tetralogy of Fallot, ventral septal defect, and atrial septal defect). Moreover, no association was found with any malformation when exposure occurred in the year before conception or during pregnancy, nor was there any relationship when exposure was stratified by specific estrogen or progestin.

A large number of reports [13 are referenced] have failed to find an association between OC/progestin exposure just before or during pregnancy and congenital heart defects or other non-genital abnormalities. There have been reviews that detail the much larger collection of reports from which this sample was taken [5 are referenced] (Reproductive Toxicity, 2/01/01). Out of 168 articles initially identified, 14 studies (7 cohort and 7 case-control) involving 65,567 women, met the criteria for meta-analysis. The authors concluded that there was no association between first trimester exposure to sex hormones generally (or to OCs specifically) and external genital malformations. Thus, women exposed to sex hormones after conception may be assured as there is no increased risk of fetal sexual malformation (Raman-Wilms, 1995). Based on the findings of these studies, it was concluded that it is unlikely that fetal anomalies or developmental lags will occur because of accidental use of POPs [progestin-only pills] during

pregnancy, nor is there any hypothesized biological mechanism for such an effect (McCann and Potter 1994).

An analysis of available epidemiologic data leads the present reviewer to conclude that the use of exogenous hormones during human pregnancy has not been proved to cause developmental abnormality in non-genital organs and tissues. This conclusion is further supported by the animal laboratory data. The preponderance of evidence at this writing indicates a lack of causal association between hormonal use during pregnancy and non-genital malformation of the offspring. The quality of the epidemiologic data does not, at this time, permit a definitive conclusion that sex hormones during pregnancy may not, under as yet to be defined conditions, have some adverse effect on human prenatal development. If there are increased risks of non-genital malformations associated with the administration of certain sex steroids, the risks are very small, may not be causal, and are substantially below the spontaneous risk of malformations.

Comments are made on a report by Janerich et al. (1974) which presented data on an association of exposure to exogenous sex steroids during pregnancy and congenital limb reduction defects. 108 mothers of patients with congenital limb reduction defects who were exposed to pregnancy tests, supportive hormone therapy, or oral contraceptives and 108 mothers of normal controls were investigated. Among mothers with malformed children, 14% had a history of exposure whereas 4% of control mothers were exposed to sex hormones ($p < .05$). 11 of the 15 affected children were male and their mothers had received orally administered sex hormones. Shortcomings of the study were noted. However, it was recommended that women who conceive while taking the pill be informed of the possibility of a sevenfold (7X) increase in risk of limb reduction defects from a frequency of .9 per 10,000 births to one of 6.9 per 10,000 births (Lanman and Jain, 1974).

The other authors found an overall increase in anomalies following progestogen exposure; limb reduction defects contributed to this increase (Greenberg and colleagues, 1977).

In a case control study that examined the relationship between congenital malformations in offspring and maternal exposure to oral contraceptives around the time of conception, there

were 1370 with congenital malformations and 2968 healthy control infants. Maternal oral contraceptive use was unrelated to malformations considered as a whole whether exposure last occurred in the year before conception [odds ratio = 0.9, p=0.25] or during pregnancy [odds ratio = 1.3, p=0.30]. Exposure during pregnancy doubled the risk for some specific diagnoses, including certain limb defects, but these increases were not statistically significant. Exposure to specific oestrogens or progestogens was also unrelated to the occurrence of malformations. There was a suggestion that women who both smoked more than 20 cigarettes per day and used oral contraceptives during pregnancy were more likely to deliver a malformed infant than were women who neither used oral contraceptives nor smoked during pregnancy (Bracken et al., 1978).

Some study indicate that the high level of vitamin A (retinol) found in the serum or plasma of women taking oestrogen-containing oral contraceptives may constitute a teratogenic hazard to women who become pregnant during, or shortly after stopping treatment with oral contraceptives on the limbs development. This was suggested to be due to Retinol in human blood is predominantly bound to a specific transport-protein known as the retinol-binding protein (RBP) (Gal, 1971).

4.2 Oral Contraceptives and Mutagenicity

A deleterious agent may cause abnormalities not only by teratogenic mechanisms but also by inducing mutations in individual germ cells. A child subsequently conceived with that germ cell would be anomalous. Both chromosomal and gene mutations have been claimed to result from sex hormone exposure (Carr, 1970).

4.2.1 Oral Contraceptives and Numeric Chromosomal Abnormalities

Earlier hormone exposure could induce chromosomal abnormalities is not an unreasonable hypothesis. Oocytes resting in dictyotene of meiosis I until ovulation might complete meiosis sluggishly as result of prior hormone exposure, resulting in numeric abnormalities of the chromosomes.

Such concern was originally raised by Carr (1970), who reported an increase in chromosomally abnormal abortuses among women previously using oral contraceptives. In such cases, 48% of abortuses were chromosomally abnormal, compared with 22% in controls; polyploidy was

responsible for most of the excess. However, these observations were not confirmed by later studies. In the study of Boué and Boué (1973), 16% of abortuses from 243 prior contraceptive users had abnormal complements (16% polyploidy), compared with 63% (18% polyploidy) of 604 controls. Other study found a slight excess of abnormal complements in previous oral contraceptive users (61% versus 49%). The increase was due to monosomy X and structural abnormalities, not to polyploidy as Carr (1970) had described (Lauritsen, 1975). Another authors observed abnormalities in 32% of 524 prior users, compared with 26% in 428 controls (Alberman et al., 1976). Similar differences by Dhardial et al., (1971) were likewise statistically insignificant. In the similar study, Klinger et al., (1976) found a higher frequency of abnormalities in induced abortions in prior contraceptive users (1%) than in controls (0.5%), but the difference was not statistically significant.

The findings that no study could confirm Carr's initial findings (1970) are especially noteworthy because any of several biases might be expected to increase anomaly rates highly in exposed groups. For example, those who induced abortuses are more likely to exist in the control group than in past users of oral contraceptives, who are more likely to be attempting to achieve a pregnancy. In fact, this may prove to be the explanation for the unusually low (22%) frequency of chromosomally abnormal abortuses in Carr's controls, because that study was conducted prior to the legalization of abortion in North America.

Other evidence supports their thesis that oral contraceptives do not induce numeric chromosomal abnormalities. If prior contraceptive use results in chromosomal abnormalities, the frequency of spontaneous abortions in previous users should be increased substantially because 50% to 60% of first-trimester abortuses have cytogenetic abnormalities. An increased incidence of Down syndrome would also be expected because the same cytologic mechanism (nondisjunction in maternal meiosis I) is usually responsible for trisomy in abortuses and liveborns. Pardthaisong et al.,(1988) found a statistically significant association between DMPA for contraception and chromosomal abnormalities (RR, 5.5; $p < .05$). Last use of DMPA occurred from 7 to 13 months before conception in three of four cases; the other mother of an infant with Down syndrome conceived while using DMPA. No association was noted among oral contraceptive users. Those authors stated that the positive association was more likely due to

chance effects than causal association. In their case-control study, Bracken et al., (1978) found no increased incidence of Down syndrome among women who used oral contraceptives up to 12 months before conception. Moreover, follow-up of prior oral contraceptive users has failed to show an increase in Down syndrome.

In summary, initial concern generated by Carr's observation (1970) of an excess of polyploid abortuses in prior oral contraceptive users has dissipated. The consensus is that neither progestins nor estrogens predispose to chromosomally abnormal abortuses.

4.2.2 Oral Contraceptives and Structural Chromosomal Abnormalities

The study of Simpson, 1985 in vitro claimed increased chromosomal breakage in lymphocytes of contraceptive users. Often such studies are performed without blind analysis. However, there are other positive lines of reassurance. No increase in structural chromosomal abnormalities was reported in the abortus studies already cited. Structural chromosomal abnormalities would also result in anomalous liveborns, and they have already concluded that no increase in the overall anomaly rate exists (Boué, 1973; Lauritsen, 1975; Alberman and Klinger, 1976).

In conclusion, the possibility that children of women exposed to oral contraceptives have an increased frequency of structural chromosomal abnormalities seems remote.

4.2.3 Oral Contraceptives and Gene Mutations

Could progestin exposure result in mutations responsible for Mendelian or polygenic/multifactorial disorders? To evaluate this hypothesis, each locus should ideally be assessed for mutability. This is mathematically impossible, even if a large increase occurs over the baseline mutation rate of 10^{-5} to 10^{-6} locus/gamete/generation. As a result, studies can generally offer only a comparison between total anomaly rates in women exposed and not exposed to hormones. Anomalies are mendelian, polygenic/multifactorial, chromosomal, or environmental in etiology. This etiologic heterogeneity diminishes the power of available conclusions. Fortunately, available data are sufficiently reassuring that more refined investigators would not be expected to yield contrary results. Not a single Mendelian disorder has increased in frequency since introduction of oral contraceptives (Harlap, 1985 and WHO, 1981).

An indirect method of researching gene mutation rates involves analysis of the gender ratio. Induction of lethal X-linked recessive mutations decreases the proportion of liveborn males; thus, lethal mutations at any X-linked loci contribute to a decreased male/female gender ratio. In fact, the larger cohort studies provide no evidence of an altered gender ratio, refuting several earlier studies of smaller sample size (Gal, 1967).

Finally, neither progestins nor estrogens are representative of classes of compounds plausibly implicated as mutagens. Progestins fail to yield mutations in the Ames test (1979), nor is there evidence that progestins induce mutagenic effects (*e.g.*, cancer) later in life.

4.3 The teratogenic effects of the Oral Contraceptives in the development of limb based on the animal models

As some studies of animal models show, for over 30 years various combinations of synthetic estrogens and progestins have been used in OCs. Ethinyl estradiol (EE) and norethindrone (NA) alone or in combination, possess low acute and chronic toxicity. These agents are not teratogenic when given in combination. Overall, the animal data demonstrates that long-term exposure to EE and NA formulations pose very little health risk (Maier and Herman, 2001).

Normal 215 adult inbred SWR/J male and female mice, 8-10 weeks old and weighing 25-30 g, were used in a study conducted by Ashmaoui and Abou-Tarbush, (2000). In each box, 4-5 females were caged together with a single male. All females were checked daily for the presence of copulatory plug (CP), which represents day 0 of gestation and the pregnant females were placed in separate cages.

Pregnant females were weighed when CP was detected, and were divided into 7 groups according to the doses calculated by Paget and Barnes (1964) as follows: GI 0.024L/0.0048E, GII 0.12L/0.024E, GIII 0.24L/0.048E, GIV 0.48L/0.096E, GV 0.72L/0.144E, GVI 0.96L/0.192E and GVII 1.20L/0.24E of EE in distilled water, and control group of animals receiving equivalent volume of distilled water only. Pregnant females received their doses from day 7 to day 12 of pregnancy. On day 17 of gestation, pregnant females were killed by cervical dislocation, then the abdominal wall was opened and both uterine horns were promptly exposed. The number of resorbed and intact fetuses was counted and recorded. Live fetuses were carefully examined under a stereoscopic microscope for gross malformations. The data were statistically analyzed

using the Students' t-test and a 2X2 contingency table (χ^2) for the actual numbers obtained according to Sokal and Rohlf (1981).

Table 3 Effects of various dose levels of Microginon-30 on fetus taken on day 17 of pregnancy in SWR/J mice (modified from Ashmaoui and Abou-Tarbush, 2000).

Group	Doses (mg/kg)	No. of female used	No. of Implantation sites (Mean +SE)	No. of Live fetus (Mean +SE)	No. of resorption(%)	Live fetal Body Wt.(g) (Mean+SE)	No. of abnormalities observed
	control	25	12.00+0.36	11.56+0.34	11(3.67)	1.00+0.015	None
I	0.024L/0.0048E	15	11.53+0.38	11.13+0.39	6(3.47)	0.99+0.033	None
II	0.12L/0.024E	15	11.13+0.40	10.40+0.52 *	11(6.59)	0.98+0.022	None
III	0.24L/0.048E	25	11.28+0.39	10.80+0.41	12(4.26)	1.05+0.012	None
IV	0.48L/0.096E	25	11.28+0.27	10.08+0.30 **	30(10.64))**	1.01+0.020	1H;3Ex
V	0.72L/0.144E	25	10.96+0.36*	10.20+0.43 *	19(6.93)	0.97+0.018	None
VI	0.96L/0.192E	25	11.24+0.38	10.16+0.42 **	27(9.61) **	0.98+0.017	None
VII	1.20L/0.24E	35	10.79+0.25**	6.26+0.77* *	165(42.97))**	0.87+0.043 **	1Ex;1T

*statistically different from the control at P<0.05

** statistically different from the control at P<0.01

L=Levonorgestrel

E=Ethinylloestradiol

T=Abnormal tail

Ex=Exencephaly

H=Abnormal hind limb

NB: Microginon 30 is a combined OCs, containing 0.15 mg of L and 0.03 mg of E, which is administered in the usual 21/7 day regime, but starting the first course on day 1 of menstrual cycle.

The effects of hormonal contraceptives on fetuses obtained from the pregnant mice are shown in Table (3). The oral administration of Microginon 30 in doses 0.12L + 0.02E (GII), 0.72L + 0.144E (GV) resulted in a decreased mean number of living fetuses (10.40 + 0.52 and 10.20 + 0.43), respectively. These results are statistically significant and are highly in GIV (10.08 + 0.30), GVI (10.16 + 0.42) and GVII (6.26 + 0.77) compared to control (11.56 + 0.34).

On the other hand, data in Table (3) indicate that the number of implantation sites was not greatly affected using different doses of Microginon 30 except in GV (10.96 + 0.36) and GVII (10.79 + 0.25) compared to control (12.00 + 0.36) which were significantly decreased.

The differences in the total resorption number between control [11 (3.67%)] and treated groups are highly significant in GIV [30(10.64%)], GVI [27(9.61)] and GVII [165(42.97%)]. But in group I, II, III, and V there is no significant difference compared to the control.

Also, statistical analysis of embryo body weights indicated that there was a highly significant difference between the mean value of the control group (1.00 + 0.01g) and GVII (0.87 + 0.43g). However, there is no significant difference between all other treated groups and the control. Morphological examination of fetuses showed in some of them abnormal hind limb, abnormal tail and exencephaly (Fig. 3). This result was clear in groups IV and VII.

The results of this study revealed that Oral Contraceptives exert deleterious effect on the development of mouse embryos. Kricker et al. (1986) reported that the use of an Oral Contraceptive during pregnancy was associated with a significantly increased risk of congenital limb defect.

Abortion attempts with large doses of estrogen, progesterone contraceptives have more been associated with malformed children (Wilson and Brent, 1981). This abnormality was observed in this study, and also high rate of resumption, which may be due to chromosomal abnormalities. Carr (1967) suggested the presence of chromosome abnormalities in 6 out of 8 spontaneous abortions from women who become pregnant after taking an Oral Contraceptive. He found a striking increase in triploidy, which he considered highly significant.

The other authors found the frequencies of abortion, neonatal death and prematurity to be significantly higher in case of diethylstilbestrol (DES). Duration of pregnancy, prematurity and neonatal death are not independent entities (Samuel and Dennis, 1979). The observation of

analysis of Bracken (1990) provides very strong evidence against the hypothesis that exposure to Oral Contraceptive in pregnancy is related to risk malformations in live born neonates.

On the other hand, in the study of Logan et al. (1989) on the effect of synthetic progestins, they found that Oral Contraceptives have potential implications for developing embryos in female mice who received the Contraceptive during early pregnancy. They assessed the effect of the progestin norgestrel on the developing pre-embryo mice and concluded that norgestrel at the dose tested (4.0 mg/ml) has no acute adverse morphological effects on mouse pre-embryo.

The result of this study, agree with Kricker et al. (1986), in their study of the congenital limb deficiencies after using an Oral Contraceptive during pregnancy of the mothers. This was associated with an increased risk of limb defect in the children. Infants were more likely to have been conceived, while the mother was using an Oral Contraceptive, especially when the deformity was a transverse or amputation type.

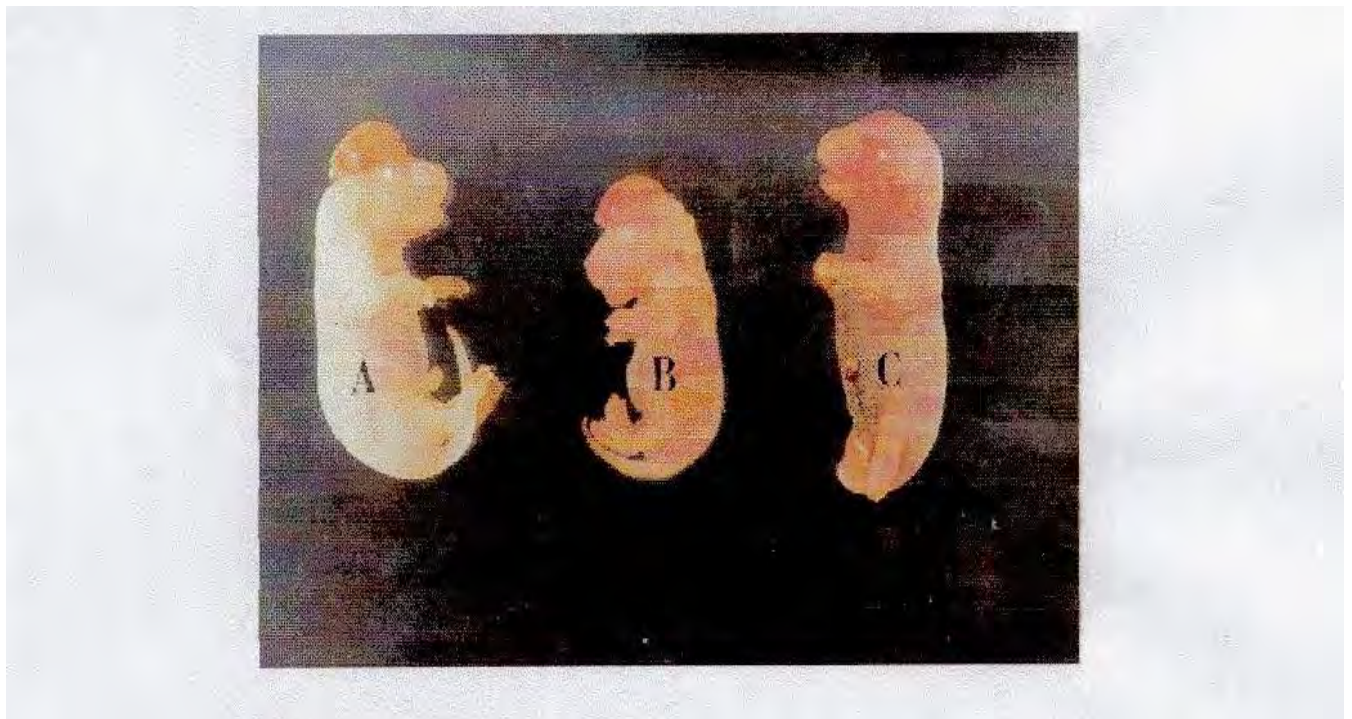


Fig. 3: Fetuses obtained on day 17 of pregnancy from female mice treated with Microginon to showing exencephaly (A,B) and abnormal hind limb and tail (C) (Ashmaoui and Abou-Tarbush, 2000).

In the study by Bracken (1990), the typical risk for congenital heart defects was 1.06 (92% confidence interval 0.72, 1.56) and for limb reduction defects, 1.04 (95% confidence interval 0.30, 3.55). This lack of an association between Oral Contraceptives and birth defects in this study agrees with the results of most of designed case control studies, which gave them a conclusion that Oral Contraceptive administered to female mice during pregnancy could induce dangerous effects on fetuses. Whereas caution must be exercised during pregnancy and women should avoid any OCs in that period.

In summary, most retrospective studies and some cohort studies have failed to show an association between progestogen exposure and limb reduction defects even if there is increased risk of congenital malformations.

5. Conclusion

Oral contraceptives are widely used and are generally safe and effective for many women. Oral contraceptive contains two synthetic steroid hormones, namely ethinyl estradiol (EE) and progestin.

The most noteworthy finding in the present review is the absence of an association between oral contraceptive use in or just before pregnancy and limb reduction defects. This association has been described in many separate studies although the findings show some variation. Various explanations for these discrepancies can be proposed. Negative findings may be random. Therefore, caution must be exercised during pregnancy and women should be advised to avoid any types of oral contraceptives in that period.

However, in some study it was indicated that women who conceive while taking the pill were with the possibility of a sevenfold (7X) increase in risk of limb reduction defects and in others the risk was doubled, but statistically it has no significant.

A synergistic reaction between exposure to oral contraceptives and heavy maternal smoking on the early fetus might also explain some of the conflicting reports in the literature. Studies which included relatively more women who were heavy smokers would be more likely to find a positive association than would studies of women who were generally not heavy smokers.

Three generalizations concerning oral contraceptives in teratogenesis have emerged. First, they are stage dependent. Second, the teratogenic response for each oral contraceptive is dose dependent. Third, some oral contraceptives are more potent (especially third generation) than others.

Combined oral contraceptive pill in the short term reduces many of the troublesome side effects associated with menstruation. In the long term, the risk of cancer of the ovary, uterus and probably also of the colon and rectum is reduced. Long-term follow-up studies have found no evidence of increased or decreased mortality in women who have taken a combined oral contraceptive. Societal benefits also increased from use of OCs, such as reduced disability due to dysmenorrhea and menorrhagia.

It might be difficult to recognize the signs and symptoms of pregnancy if women become pregnant while taking the minipill (progestin-only birth control pill) because nausea, breast tenderness and irregular menstrual bleeding common signs and symptoms of pregnancy are also possible side effects of the minipill.

As a precaution, women should stop taking birth control pills if they suspect that they are pregnant. Until the pregnancy is confirmed or ruled out, they should use another method of birth control such as condoms. If they are concerned because they took birth control pills before they knew that they were pregnant, be assured that there's little risk. It's always important to discuss any medication use during pregnancy with their health care provider.

6. Recommendation

Since currently there is inadequate data with regard to teratogenic effects of oral contraceptive further studies are required to know about its safety.

In addition there exists no study with regard to congenital limb reduction defects in many countries, including Ethiopia and the study regarding the effects of oral contraceptives on congenital anomalies should be broadened to include these countries.

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