REVIEW ARTICLE

Factor Effecting Infertility and Childlessness Correlated to Anovulatory Menstrual Cycles in Women

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ABSTRACT

Infertility and childlessness are one of the most important health problems in women. The objective of present review was to identify the infertility associated factors with anovulatory menstrual cycles in women. Infertility is defined as the inability to conceive after one to two years of unprotected intercourse. In the general population, conception is expected to occur in 84% of women within 12 months and in 92% within 24 months. According to the European Society of Human Reproduction and Embryology Classification, infertility is defined as the diminished ability, or the inability to conceive. Infertility is also defined in specific terms as the failure to conceive after at least one year of intercourse without contraception. One of the most important and underappreciated reproductive health problems in developing countries is the high rate of infertility and childlessness. The inability to procreate is frequently considered a personal tragedy and a curse for the couple, impacting on the entire family and even the local community. In many cultures, womanhood is defined through motherhood and infertile women usually carry the blame for the couple's inability to conceive. Moreover, in the absence of social security systems, older people are economically dependent on their children. Childless women are frequently stigmatized, resulting in isolation, neglect, domestic violence and polygamy. Several studies have demonstrated that anxiety has a detrimental effect on fertility and that reduction of anxiety increases pregnancy rate. If any menstrual problem is observed, doctor should be concerned immediately. Unhygienic home deliveries should be avoided to prevent infection leading to secondary infertility due to tubal blockage. Balance diet and exercise increases insulin sensitivity, which improves ovarian function and the chance of conception.

Key words: Infertility, menstrual cycles, childlessness

INTRODUCTION

Anovulation can arise from a number of causes, ranging from diet and exercise to complex disruptions in the relationships between tiny glands in the brain that control our most basic functions. Some causes are relatively easy to identify, whereas others are much more difficult. Hormonal imbalances are the most probable cause of anovulatory cycle. A prolonged, strenuous program of exercise, such as running, can interfere with the ovulatory cycle by suppressing the output of hormones called gonadotropins from the hypothalamus in the brain. Anxiety and other forms of emotional stress can also take their toll on normal ovulation (Vilos et al; 2001). The disorder may also result from eating disorders, hypothalamic dysfunction, hyperprolactinemia, polycystic ovary syndrome, luteal phase defects, or tumors of the pituitary gland adrenal gland or ovaries. Other causes of anovulatory cycles are primary ovarian failure, resistant ovary syndrome and autoimmune oophoritis (Millie, 2005). Another possible contributor to anovulation is the long-term use of certain medications. Steroidal oral contraceptives (the Pill) are sometimes responsible. These drugs work by intentionally disrupting the hypothalamic-pituitary-ovarian axis, suppressing ovulation and thereby preventing pregnancy. For women using long-acting injectable steroid contraceptives (Depo-Provera), it appears likely that the longer the contraceptive is continued the more likely it is that amenorrhea will result. Once patient know the probable causes of anovulation, you must take care to avoid any of these, especially if you have a history of fertility problems. Avoid any strenuous exercise without consultation and do not attempt to try out fad diests as these may lead to anovulatory cycles. Learn to manage stress and develop a healthy lifestyle to keep this disorder at bay

Prevalence of Infertility in Developing Countries: It is generally believed that more than 70 million

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couples suffer from infertility worldwide (Fathalla, 1992; Boivin et al., 2007). In a study Boivin et al; (2007) reported that the 12-month prevalence rate ranges from 6.9 to 9.3% in less-developed countries. Substantial geographical differences in the prevalence are noted, and these differences are largely explained by different environmental, cultural and socioeconomic influences.In sub-Saharan Africa, the prevalence differs widely from 9% in the Gambia (Sundby et al., 1998) and 11.8% in Ghana (Geelhoed et al., 2002) compared with 21.2% in northwestern Ethiopia (Haile, 1990) and between 20 and 30% in Nigeria (Larsen, 2000). Even less data are available from Asia and Latin-America, but a report compiled by the World Health Organization (WHO) indicated that the prevalence of infertility in these regions fell within the globally expected range 8 –12% of couples of reproductive age and was thus lower as compared with African countries (World Health Organization, 1991). In a large study performed by the WHO Task Force on the Diagnosis and Treatment of Infertility, 8504 infertile couples in 33 different countries were examined through a standard approach in all participating centers (Cates et al., 1985). In Africa, over 85% of women had an infertility diagnosis attributable to an infection compared with 33% of women worldwide. In another study from Sub-Saharan Africa, a history of sexually transmitted diseases (STDs) was reported by 46% of participating men (Gerais and Rushwan, 1992). A study of 5800 couples in 33 World Health Organization centres in 25 countries showed that almost 50% of the African couples and 11–15% of other patients in other parts of the world had infectious tubal disease (Sciarra, 1994). Individual studies from Nigeria, South Africa and Egypt have reported prevalence rates of tubal factor infertility ranging from 42 to 77% (Ikechebelu et al., 2003).

Anovulatory Menstrual Cycle: An anovulatory cycle is a menstrual cycle in which ovulation fails to occur. It is a disorder of the menstrual cycle in which a woman does not release an egg for fertilization every month, although she menstruates. Hormonal imbalance is the most probable cause of anovulatory cycle. The duration of these cycles can vary form average of 25-35 days up to 35-50 days (Nelson, 1993). Anovulatory cycles accounts for 21% cases of infertility. But these cycles are most common during adolescence and in years before menopause. Polycystic Ovarian Syndrome accounts for 36% of anovulatory cycles, premature ovarian failure 23% and hyper prolactinemia 17% (Balen et al., 1993).

Women with anovulatory cycles do not experience symptoms at the time of ovulation. These symptoms includes rise in the body temperature, an average 0.4 to 0.6 degrees Fahrenheit (0.2 degrees Celsius), pain in the lower abdomen and change in the chemical composition of the cervical mucus, which becomes clear, stretchable and sticky (Guraya & Dhanju, 1992). Symptoms of anovulation include abnormal or erratic basal body temperature (BBT) and few or absent pre-menstrual symptoms. These cycles are usually associated with dysfunctional uterine bleeding. Anovulatory cycle have no corpus luteum formation. Progesterone is not produced, the endometrium continues to proliferate under the influence of unopposed estrogen, eventually this endometrium is shed in an irregular manner that might be prolonged and heavy (Millie, 2005). Anovulatory bleeding causes a higher risk of endometrial hyperplasia (Vilos et al; 2001).

The patients with a normal menstrual cycle (3-6 weeks) had a single measurement of serum progesterone concentration timed to occur 5-10 days before the ensuing menstrual period in two or three cycles. The patients were classified as ovulatory, if the progesterone value was at least 30 n mol/l (9.4 ng/ml) in two of the three cycles. Conversely, those with a value less than 30 n mol/l in two cycles were classed as having luteal deficiency, which for practical purposes was taken to be a form of ovulatory failure (Hull et al., 1982).

Patho-physiology: Luteinizing hormone (LH) is the physiologic signal necessary for ovulation, which is mediated by a concomitant surge in estrogen. As the follicle grows through accumulation of follicular fluid, the cohort of granulosa cells acquire the necessary receptors to respond to LH with increased formation of cyclic adenosine monophosphate (cAMP). Speroff, et al. (1999) reported that approximately 16-24 hours after the LH peak, ovulation occurs with the extrusion of a mature graafian follicle and the formation of the corpus luteum. These events occur due to well-coordinated interplay between hormones and their appropriate receptors and proteolytic enzymes and prostaglandins acting in concert with one another, all directed by the HPO (Hypothalamic-Pitutary-ovarian) axis. The system is so sensitive that even the slightest alteration in any of these factors can disrupt its fluidity and lead to anovulation. When problems arise at any of the many different levels involved in the normal menstrual cycle, it is sometimes helpful to separate the levels by organ system. The hypothalamus and the anterior pituitary can be considered the neuroendocrine components by virtue of their proximity to each another, while the ovaries are a separate compartment. The third aspect that can be defective is the signaling process that occurs between these two areas.

The initial stimulus must come from the hypothalamus in the form of gonadotrophin-releasing hormone (GnRH); this decapeptide must be secreted

in a pulsatile fashion within a critical range. For example, sexual maturity is not attained until the onset of regular ovulatory cycles, which may take months to years to occur. This maturation process is orchestrated by a neuroendocrine cascade and modified by autocrine and paracrine events in the ovaries, in which GnRH is the principal mediator (Spence, 1997).

Any alteration in the GnRH pulse generator alters the hormonal milieu necessary for gonadotropin secretion and eventual response at the level of the ovary. Several entities (e.g., hyperprolactinemia) are known to cause this type of dysregulation. Increasing levels of prolactin can cause a woman to progress from a deficient luteal phase to overt amenorrhea, usually associated with complete GnRH suppression. More common causes of dysregulation include stress, anxiety, and eating disorders, which are also associated with an inhibition of normal GnRH pulsatility through excessive hypothalamic activity of corticotrophin-releasing hormone and stimulation of beta-endorphins (Yen et al., 1999).

It is still unknown that how polycystic ovary syndrome (PCOS) is associated with anovulatory cycles has not been completely elucidated? It is thought to be two associations with this disease could be responsible for its development. The first is the persistent elevation of LH levels in these patients; the second is the apparent arrest of antral follicle development at the 5-10 mm stage and consequent failure to enter the pre-ovulatory phase of the cycle (Frank et al., 1998). This evidence indicates that the disturbance is mainly a central defect that initiates the cascade of events leading to its onset. Similarly, any condition, whether primary or secondary, that results in either a persistent elevation or an insufficient attainment of estrogen levels can inhibit ovulation through a disruption of the mechanisms that induce the LH surge. In order to achieve the corresponding changes within the cycle, estradiol levels must rise and fall appropriately (Speroff et al., 1999).

Oocyte Maturation: One of the final steps in the development of an oocyte is maturation. Oocyte maturation is defined as a re-entry into meiosis that occurs just prior to ovulation and subsequent fertilization Oocytes within the ovary are arrested in prophase, I. of meiosis until the gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), stimulate follicular growth and development, which then triggers the resumption of meiosis up to metaphase, II. Oocytes are subsequently again held in meiotic arrest until fertilization, when meiosis is completed (Albertini andCarabatsos et al., 1998).

Oogenesis: During human fetal development, primordial germ cells originate in the dorsal endoderm of the yolk sac, close to the allantoic evagination. By weeks 4 to 5, these cells migrate through the hindgut before settling in the urogenital ridge (Dizerega and Hodgen, 1981). By week 25 of gestation, approximately 7 million oogonia have been formed by mitosis and developed into primary oocytes (Griffin and Ojeda, 2000). At this stage, a single layer of flattened epithelial cells surrounds the primary oocyte to form a primordial follicle. The first steps of meiosis- I occur in the primordial follicle, including the replication of DNA and arrest in the diplotene stage of prophase -I.

Meoitic Arrest: One of the most important intracellular signaling molecules believed to be responsible for maintaining meiotic arrest is cAMP (Conti et al., 2002). In general, intracellular cAMP homeostasis is regulated by two important groups of enzymes: the adenylyl cyclases (ACs), which generate cAMP; and the phosphodiesterases (PDEs), which metabolize cAMP. Most of the well-characterized ACs are regulated by G proteins that either promote (Gαs) or inhibit (Gαi) their activity (Freissmuth et al., 1989).

Ovarian anatomy & Gap junction: The mammalian follicle consists of several outer layers of theca cells that play a prominent role in ovarian steroid-genesis. These theca cells surround layers of outer mural granulosa cells, which in turn encompass layers of inner cumulus granulosa cells. These cumulus granulosa cells then form close contacts with the oocytes via gap junctions. Gap junctions are composed of proteins from the connexin family; connexin 43 (Cx43) is the primary member in the ovary. Cx43-containing gap junctions appear to be necessary for expansion of granulosa cells during follicular growth (Ackert et al., 2001) as well as for oocyte development. Likewise, the role of gap junctions in transferring nutrients and metabolic precursors to mammalian oocytes is well established (Herlands and Schultz, 1984).

Growth Factors: Although exposure of ovarian follicles to LH leads to oocyte maturation, neither oocytes nor surrounding cumulus granulosa cells contain LH receptors. Instead, the theca and mural granulosa cells express LH receptors, indicating that some paracrine factor(s) must be released by these outer cells in response to LH to promote expansion of cumulus cells and oocyte maturation (Peng et al., 1991). Although the ability of growth factors to promote cumulus cell expansion and subsequent oocyte maturation in follicle cultures has been long established (Downs et al., 1988), their physiologic role in regulating meiosis and ovulation had been uncertain. First, in addition to promoting steroid

production, LH triggers the release of growth factors from the theca and/or mural granulosa cells. These growth factors then act in a paracrine fashion to stimulate inner cumulus granulosa cell expansion, which is followed by disruption of oocyte-granulosa cell contacts, oocyte maturation, and eventual ovulation.

Factors Affecting Ovulation

Effect of Carbohydrates: Jorge, et al. (2007) reported that use of different form of carbohydrates also influence fertility. Eating lots of easily digested carbohydrates (fast carbohydrates), such as white bread, potatoes and sugared sodas, increase the odds that one find struggling with ovulatory fertility. Eating a lot of rapidly digested carbohydrates will continually boost the blood sugar and insulin levels. These increased levels will disrupt the balance of hormones needed for reproduction. The ensuing hormonal changes will affect the ovulation. The more fast carbohydrates in the diet, the higher the glycemic load. Women in the highest glycemic load category were 92 % more likely to have had ovulatory infertility than women in the lowest category. Choosing slowly digested carbohydrates (slow carbohydrates) that are rich in fiber can improve fertility. Whole grains, beans, vegetables and whole fruits, whole of which are good sources of slow carbohydrates. In other words, eating a lot of easily digested carbohydrates increases the odds of ovulatory infertility, while eating, more slow carbohydrates decreases the odds.

Effect of Trans Fats: Today there are no definitive indications of any diet factors influencing human fertility; however a recent study suggests that trans fats increase a woman's risk of infertility. Trans fat is the common name for a type of unsaturated fat with trans–isomer fatty acid (s). Trans fat may be monounsaturated or polyunsaturated. Another name for trans fat is “partially hydrogenated oils”. Trans fats can be found in vegetable shortenings, some margarines, crackers, cookies, snack foods, and other foods made with or fried in partially hydrogenated oils. It is not clear how the trans fats affect ovulation, but they may affect sensitivity to insulin, which is known to play a role in infertility problems. The study was performed by the researchers from the Harvard School of Public Health. Their studies showed that each 2% increase in trans fat consumption as a replacement for carbohydrates resulted in a 73% greater risk of ovulation related infertility (Chavarro, et al; 2007).

Effect of Dairy Fat Products: Eating two or more servings of low-fat dairy products a day, which could include a portion of cottage cheese and a low-fat yoghurt, increased the risk of infertility due to lack of ovulation by 85 % , the researchers found. But women who ate at least one serving of high-fat dairy food a day cut their risk of infertility from this cause by 27 % (Jeremy, 2007).

Effect of Vitamin Supplements: In analyses adjusted for age and calendar time, use of multivitamin supplements was associated with a decreased risk of ovulatory infertility in a dose-dependent manner. Multivitamin users had approximately one-third lower risk of developing ovulatory infertility when compared to non-users (Jorge, et al; 2008).

Effect of Light: Forty years ago it was asserted that light might regulate the menstrual cycle and ovulation (Dewan, 1967). A series of six studies—one in the Boston area, four in San Diego, and one in Novosibirsk (1965–1995)—have shown that the menstrual cycle shortened in women with long menstrual cycles after bedside light administered overnight around the days of presumed ovulation (Putilov et al; 2002). Two subsequent studies found an increased rate of ovulation determined with a home dip-stick test detecting an ovulatory surge of LH in urine (Rex et al; 1997 and 1999).

Effect of Iron Deficiency: Iron deficiency is the most prevalent nutritional deficiency worldwide. In the United States, women of child bearing age are at increased risk of this condition (Centers for Disease Control and Prevention (CDC). 1999-2008). In addition, iron supplements have been associated with a lower prevalence of iron deficiency among women of reproductive age (Cogswell et al; 2003). The role iron status may have on reproduction is further highlighted by studies regarding iron-transporting proteins in key ovarian cells.

The presence of transferrin and its receptor in granulosa cells and oocytes has been documented in several studies. More recently, it has been reported that granulosa cells can synthesize transferrin, which may be translocated to the oocytes (Briggs, et al; 1999). Although it is possible that transferrin and transferrin receptor are redundant in the ovary or do not play an important role in local iron metabolism, it has been suggested that these proteins are essential for ovum development and are required to support the increased iron demand of the developing follicle (Aleshire et al., 1989).

Effect of Protein: The protein in the diet may influence blood sugar, sensitivity to insulin and the production of insulin - like growth factor–1. All of which play important roles in ovulation. The study was performed by the researchers from the Harvard School of Public Health. The participants were group according to their average daily protein intake. The women in the highest protein group were 41 percent more likely to have reported problems with ovulatory infertility than women in the lowest protein group (Healy et al; 1994).

Effect of Life Style: Lifestyle factors are behaviors and circumstances that are, or were once, modifiable and can be a contributing factor to sub fertility. It has been estimated that approximately 15% of the population in industrially developed countries are affected (Healy et al; 1994). There is an increasing body of evidence that lifestyle factors can have an impact on reproductive performance**.**

a). Weight: Obesity and low body weight can affect on reproductive function by causing hormone imbalances and ovulatory dysfunction. Abnormal weight is usually defined as a high BMI (kg/m^2) of ≥25 and a low BMI of <20 and the effects of abnormal weight have been reported in several papers (Hassan and Killick, 2004; Norman et al; 2004). The results of an Australian study of 87 obese (BMI 30) infertile women attending a weekly programme to promote lifestyle changes demonstrate that a relatively small amount of weight loss (average of 6.5 kg) was associated with resumption of ovulation (Clark et al., 1998). The relationship between obesity and reproductive problems is complicated. However, insulin resistance and estrogen production from fat cells can affect the ovaries and prevent ovulation.

b). Smoking: Studies have demonstrated that smoking in women significantly decreases the chance of conception (Augood et al., 1998). In the female, the constituents of cigarette smoke may affect the follicular microenvironment and alter hormone levels in the luteal phase.

c). Psychological Stress: Psychological stress may reduce female reproductive performance in various ways. The autonomic nervous system, the endocrine and immune systems have all been implicated. There is, however, a lack of clear consensus as to the definition and measurement of 'psychological stress' (Domar et al., 2000), bringing into question the nature and strength of any putative association. Given that infertility and ART (Assisted Reproductive Technologies) treatment are associated with stress (Olivius et al., 2004).

d). Exercise: Rich-Edwards et al. (2002) found that exercise was associated with a reduction in risk of ovulatory infertility. Exercise increases insulin sensitivity, which improves ovarian function and the chance of conception.

e). Female Age: By the time women reach 35 years of age, their fertility is declining. At an even earlier age, the number and quality of oocytes decrease but manifest clinically at around 35 years of age (Baird et al., 2005).

f). Alcohol: Alcohol is a known teratogen (Chaudhuri, 2000) and its consumption has been reported to decrease fertility, although the level of consumption associated with risk is unclear. Alcohol consumption at the extreme level is known to be dangerous to the unborn child (Krulewitch, 2005) but the effect at lower levels is less certain. The mechanisms by which alcohol could impair conception are unclear, but may include an alcohol-induced rise in estrogen, which reduces FSH secretion suppressing folliculogenisis and ovulation. It may also have a direct effect on the maturation of the ovum, ovulation, blastocyst development and implantation (Eggert et al., 2004).

Effect of reactive oxygen species: Reactive oxygen species (ROS) have a physiological and pathological role in the female reproductive tract. Numerous animal and human studies have demonstrated the presence of ROS in the female reproductive tract: ovaries, fallopian tubes and embryos (Suzuki, et al; 1999; Jozwik, et al; 1999). ROS is involved in the modulation of an entire spectrum of physiological reproductive functions such as oocyte maturation, ovarian steroidogenesis, corpus luteal function and luteolysis (Ishikawa, 1993).

Diagnostic Tests for Anovulatory Cycles: Anovulation can be difficult to detect. Some women have seemingly normal menstrual periods even though they are not ovulating. Most often, women who do not ovulate also do not menstruate, a disorder known as amenorrhea, or do not menstruate regularly, a condition called oligomenorrhea. Because of this, scant, erratic, short and/or painless menstrual cycles can sometimes alert a woman or her doctor about an anovulation problem. If you experience bleeding between periods for more than 2-3 cycles, you should report your doctor without delay. Too many of these anovulatory cycles can contribute to irregular bleeding, or endometrial hyperplasia. A diagnostic test for anovulatory cycles may include the following depending on other factors like your age and medical history. Serum assays, LH (on days 13 and 15 of menstrual cycle, to detect midcycle peak)**,** FSH**,** Progesterone**,** Prolactin**,** DHEA-SO4**,** Testosterone and SHBG (for obtaining Free Androgen Index [FAI] or calculated free testosterone levels)**,** CT scan or MRI scan of pituitary and hypothalamus**.** Endometrial biopsy**,** Ovarian biopsy**,** Specific antibody tests (WHO 1987 and 1991).

Treatment for Anovulatory Cycles: Treatments for anovulatory cycles vary based on the underlying cause of the condition and other factors like age and medical history. For many infertile women with anovulation, treatment with one or another of fertility drugs can be remarkably successful. Clomiphene citrate (Clomid) is often a good first choice for an anovulatory woman who is producing estrogen. If clomiphene alone is unsuccessful, Pergonal is added to bolster the attempts to ripen a follicle. Pergonal bypasses the natural hormone stimulation of the

pituitary on the ovary. It applies stimulation directly to the ovary, and then, once a follicle grows to sufficient size, HCG is used as the final step to release the egg. Some other treatments that can use in tandem with medication are: Medications for specific underlying conditions should used regularly, nutritional modifications, stress reduction are keys to overcome the condition. Surgery is required in the case of tumors

REFERENCES

1. Ackert CL, Gittens JE, O'Brien MJ, Eppig JJ, Kidder GM (2001). Intercellular communication via connexin43 gap junctions is required for ovarian folliculogenesis in the mouse. Dev Biol. 233: 258 – 270.
2. Albertini DF, Carabatsos MJ (1998). Comparative aspects of meiotic cell cycle control in mammals. J Mol Med. 1998; 76: 795 – 799.
3. Aleshire SL, Osteen KG, Maxson WS, Entman SS, Bradley CA, Parl FF (1989). Localization of transferrin and its receptor in ovarian follicular cells: morphologic studies in relation to follicular development. Fertil Steril 51: 444 – 9.
4. Augood C, Duckitt K, Templeton AA (1998). Smoking and female infertility: a systematic review and meta-analysis. Hum Reprod 13:1532–1539.
5. Baird DT, Collins J, Egozcue J, Evers LH, Gianaroli L, Leridon H, Sunde A, Templeton A, Van Steirteghem A, Cohen J, et al; (2005). Fertility and ageing. Hum Reprod Update 11:261–276.
6. Balen A.H, Schoham Z & Jacobs HS (1993a). Anovulation causes and consequences. In: Asch RH & stud JJW (eds) Annual Progress in Reproductive Medicine C or forth, Lancashire: Parthenon Press PP.205-34.
7. Boivin, J; Bunting, L; Collins, JA; Nygren, KG (2007). International estimates of infertility prevalence and treatment-seeking: potential need and demand for infertility medical care. Hum Reprod.22:1506 –1512.
8. Briggs DA, Sharp DJ, Miller D, Gosden RG (1999). Transferrin in the developing ovarian follicle: evidence for de-novo expression by granulosa cells. Mol Hum Reprod 5:1107–14. Cates, W; Farley, TM; Rowe, PJ (1985). Worldwide patterns of infertility: is Africa different? Lancet.2: 596 –598.
9. Chaudhuri JD (2000). An analysis of the teratagenic effects that could possibly be due to alcohol consumption by pregnant mothers. Indian J Med Sci 54: 425 – 431.
10. Chavarro J. E., Rich-Edwards J. W., Rosner B. A., Willett W. C (2007). Dietary fatty acid intakes and the risk of ovulatory infertility. American Journal of Clinical Nutrition, vol 85, pp 231-237.
11. Clark AM, Thornley B, Tomlinson L, Galletley C, Norman RJ (1998). Weight loss in obese infertile women results in improvement in reproductive outcome for all forms of fertility treatment. Hum Reprod 13: 1502 –1505.
12. Cogswell ME, Kettel-Khan L, Ramakrishnan U (2003). Iron supplement use among women in the United States: science, policy and practice. J Nutr 133:1974S–7.
13. Conti M, Andersen CB, Richard F, et al; (2002). Role of cyclic nucleotide signaling in oocyte maturation. Mol Cell Endocrinol 187:153 –159.
14. Dewan EM (1967). On the possibility of a perfect rhythm method of birth control by periodic light stimulation. Am J Obstet Gynecol 99: 1016 -1018.
15. Dizerega GS, Hodgen GD (1981). Folliculogenesis in the primate ovarian cycle. Endocr Rev 2: 27 – 49.
16. Domar AD, Clapp D, Slawsby EA, Dusek J, Kessel B, Freizinger M (2000). Impact of group psychological interventions on pregnancy rates in infertile women. Fertil Steril 73:805 – 8. Downs SM, Daniel SA, Eppig JJ (1988). Induction of maturation in cumulus cell-enclosed mouse oocytes by follicle-stimulating hormone and epidermal growth factor: evidence for a positistimulus of somatic cell origin. J Exp Zool 245: 86 – 96.
17. Eggert J, Theobald H, Engfeldt P (2004). Effects of alcohol consumption on female fertility during an 18-year period. Fertil Steril 81:379 –38.
18. Fathalla, MF (1992). Reproductive health: a global overview. Early Hum Dev. 29:35 – 42.
19. Franks S, Mason H, White D, and Willis D (1998).Etiology of anovulation in polycystic ovary syndrome. Steroids.May-Jun 63 (5-6): 306 - 7.
20. Freissmuth M, Casey PJ, Gilman AG (1989). G proteins control diverse pathways of transmembrane signaling. FASEB J. 3: 2125 – 2131.
21. Geelhoed, DW; Nayembil, D; Asare, K; Schagen van Leeuwen, JH; Roosmalen, J (2002). Infertility in rural Ghana. Int J Gynaecol Obstet. 79: 137 –142.
22. Gerais, AS; Rushwan, H (1992). Infertility in Africa. Popul Sci.12: 25 – 46.
23. Griffin JE, Ojeda SR (2000). Textbook of Endocrine Physiology, 4th ed. New York: Oxford University Press.
24. Guraya S S. Dhanju CK (1992). Mechanism of Ovulation –an overview Indian J Exp Biol 30: 958 – 967.
25. Haile, a (1990). Fertility conditions in Gondar, northwestern Ethiopia: an appraisal of current status. Stud Fam Plann. 21: 110 –118.
26. Hassan MA and Killick SR (2004). Negative lifestyle is associated with a significant reduction in fecundity. Fertil Steril 81:384–392.
27. Healy DL, Trounson AO, Andersen AN (1994). Female infertility: *causes and treatment.* Herlands RL, Schultz RM (1984). Regulation of mouse oocyte growth: probable nutritional role for intercellular communication between follicle cells and oocytes in oocyte growth. J Exp Zool 229: 317 – 325.
28. Hull MGR, Savage PE, Bromham DR, Ismail AAA,Morris AF (1982). The value of a single serum progesterone measurement in mid luteal phase as criterion of a potentially fertile cycle ("ovulation") derived from treated and untreated conception cycles. Fertil Steril 37: 355 - 60.
29. Ikechebelu, JI; Adinma, JI; Orie, EF; Ikegwuonu, SO (2003). High prevalence of male infertility in

southeastern Nigeria. J Obstet Gynaecol. 23: 657–659.
30. Ishikawa, M (1993). Oxygen radicals-superoxide dismutase system and reproduction medicine. Nippon Sanka Fujinka Gakkai Zasshi. 45: 842 – 848.
31. Jeremy Laurance (2007). 'Ice cream 'helps increase chance of pregnancy'. The Independent Health and WellBeing. The Health News, Wednesday 28, February (2007).
32. Jorge E. Chavarro, M.D, Walter C and Patrick J (2007). Fat, Carbs and the Science of Conception Print Article Newsweek, December 10, 2007.
33. Jorge E. Chavarro, MD, Janet W, and Walter C (2008). Use of multivitamins, intake of B vitamins and risk of ovulatory infertility. Fertil Steril 89(3): 668 – 676.
34. Jozwik, M; Wolczynski, S; Szamatowicz, M (1999). Oxidative stress markers in preovulatory follicular fluid in humans. *Mol Hum Reprod.* 5: 409 – 413.
35. Krulewitch CJ (2005). Alcohol consumption during pregnancy. Annu Rev Nurs Res 23:101–134.
36. Larsen, U (2000). Primary and secondary infertility in sub-Saharan Africa. International Journal of Epidemiology 29:285-291.
37. Millie Behera, Gail F Whitman – Elia Thomas Michael Price, John T. Queenan, Jr (2005). Americal Journal of obstetrics & Gynecology.
38. Nelson S (1993). OS Orgaos Sexuais Feminions: Forma, Funcao Simbol e Arquetiopo Published by Imago Editora. Riode Janeiro.
39. Norman RJ, Noakes M, Wu R, Davies MJ, Moran L, Wang JX (2004). Improving reproductive performance in overweight/obese women with effective weight management. Hum Reprod Update 10: 267 – 280.
40. Olivius C, Friden B, Borg G, Bergh C (2004). Why do couples discontinue in vitro fertilization treatment? A cohort study. Fertil Steril 81: 258 – 261.
41. Peng XR, Hsueh AJ, LaPolt PS, Bjersing L, Ny T (1991). Localization of luteinizing hormone receptor messenger ribonucleic acid expression in ovarian cell types during follicle development and ovulation. Endocrinology. 129: 3200 – 3207.
42. Putilov AA, Danilenko KV, Protopopova AY, Kripke DF (2002). Menstrual phase response to nocturnal light. Biol Rhythm Res. 33: 23 – 38.
43. Rex KM, Kripke DF, Cole RJ, Klauber MR (1997). Nocturnal light effects on menstrual cycle. J Altern Complement Med. 3:387 – 390.
44. Rex, KM.; Kripke, DF.; Cole, RJ (1999). Lack of dose-response effect of nocturnal light on menstrual cycle length. In: Holick MF, Jung EG., editors. Biological effects of light 1998: Proceedings of a symposium. Boston/London/Dordrecht: Kluwer Academic Publishers; pp. 455 – 458.
45. Rich-Edwards JW, Spiegelman D, Garland M, Hertzmark E, Hunter DJ, Colditz GA, Willett WC, Wand H, Manson JE (2002). Physical activity, body mass index, and ovulatory disorder infertility. Epidemiology 13: 184 – 190.
46. Sciarra, JJ (1994). Infertility: a global perspective. The role of pelvic infection. *ORGYN.*3: 12 – 15.
47. Spence JE (1997). Anovulation and monophasic cycles. Ann N Y Acad Sci. Jun 17 1997; 816: 173 - 6.
48. Speroff L, Glass RH, Kase NG (1999). Anovulation and the polycystic ovary syndrome. In: Clinical Gynecologic Endocrinology and Infertility. Philadelphia, PA: Lippincott Williams & Wilkins; 487 - 513.
49. Sundby, J; Mboge, R; Sonko, S (1998). Infertility in the Gambia: frequency and health care seeking. Soc Sci Med. 46: 891– 899.
50. Suzuki, T; Sugino, N; Fukaya, T; Sugiyama, S; Uda, T; Takaya, R; Yajima, A; Sasano, H (1999). Superoxide dismutase in normal cycling human ovaries: immunohistochemical localization and characterization. Fertil Steril. 72:720 – 726.
51. Vilos GA, Leng occuringnfebvre G, Graves GR (2001). Guidelines for the management of abnormal uterine bleeding. J Obstet Gynecol Can. 23 (8): 704 – 9.
52. World Health Organization. Infections, pregnancies and infertility: perspectives on prevention. Fertil Steril. 1987; 47: 944 – 949.
53. World Health Organization. Infertility: A Tabulation of Available Data on Prevalence of Primary and Secondary Infertility. Geneva: WHO (1991). WHO / MCH / 91.9.
54. Yen SC, Jaffe RB, Barbieri RL (1999). Chronic anovulation due to CNS-hypothalamic - pituitary dysfunction.In: Reproductive Endocrinology: Physiology, Pathophysiology and Clinical Management.4th ed. London, England: Elsevier Science.