2.0 REVIEW OF LITERATURE

2.1 Arsenic, a major water pollutant: Arsenic contamination in drinking water at global basis:

From the literature review it is revealed from the studies several of the countries including India where our state, West Bengal is affected remarkably by the arsenic. The regions of the world in chronic exposure at relatively higher concentrations via drinking water caused by public massive health hazards. These potential impacts on human health have been noticed in such area where arsenic percolates into ground water or mainly mining and metallurgy industrial belt or enter into surface water in volcanic deposited belt, as may be found in, India, Bangladesh, Taiwan, China, Thailand, Inner Mongolia, Chile, Argentina, Mexico, Hungary and Finland. In the United States, total non-occupational exposure to inorganic arsenic from all routes is primarily by ingestion of arsenic contaminated water and food [**Sankar & Shanker, 2014]**

Following table shown countrywide distribution of arsenic in drinking water of affected regions

Country	**As level in Groundwater (ppb)**
India	10-3200
Bangladesh	50-2000
China	50-4440
Mexico	8-620
Thailand	1->5000
USA	Up to 2600
Taiwan	10-1820

Table 2 A.I Arsenic concentration in some countries. [Chowdhury et al., 2000.]

2.1.1 Arsenic contaminated drinking water in India:

In India, a wide number of population currently exposed unacceptably higher concentration of arsenic by using ground water daily household use and agricultural purpose [**Guha Majumdar et al., 2010**]. The people of India in several areas of Uttar Pradesh, Bihar, West Bengal and wide areas of eastern part of Assam are mainly exposed by arsenic-contaminated drinking water [**Roy & Saha, 1999; Guha Majumdar et al., 2010**]. According to proposal guideline of WHO (**2001**) the recommended admissible limit of arsenic in drinking water is 0.01 parts per million (0.01mg/lit or 10μg/lit) whereas in India the level in groundwater is above the benchmark (some parts≥40 μg/lit.) [**Dey et al., 2014].**

2.1.2 Arsenic contamination in West Bengal:

From literature review up-to the end of 2017, it has been noted that 9 districts out of 16 are affected by arsenic intoxication. About 1.04 crore people in 83 blocks of 8 districts as Burdwan, Malda, Hoogly, Howrah, Mursidabad, North and South 24 Parganas, Nadia and Kolkata in our state West Bengal are hit by arsenic contamination via drinking water [**Santra, 2017**]. The total area and population of these affected areas are 38,000 km^2. The arsenic level in these regions varies from 0.02 ppm to 0.96 ppm and several of them show high concentration of inorganic arsenic containing of 0.4ppm [**Saha et al. 1999; Guha Mazumder et al., 2010**).

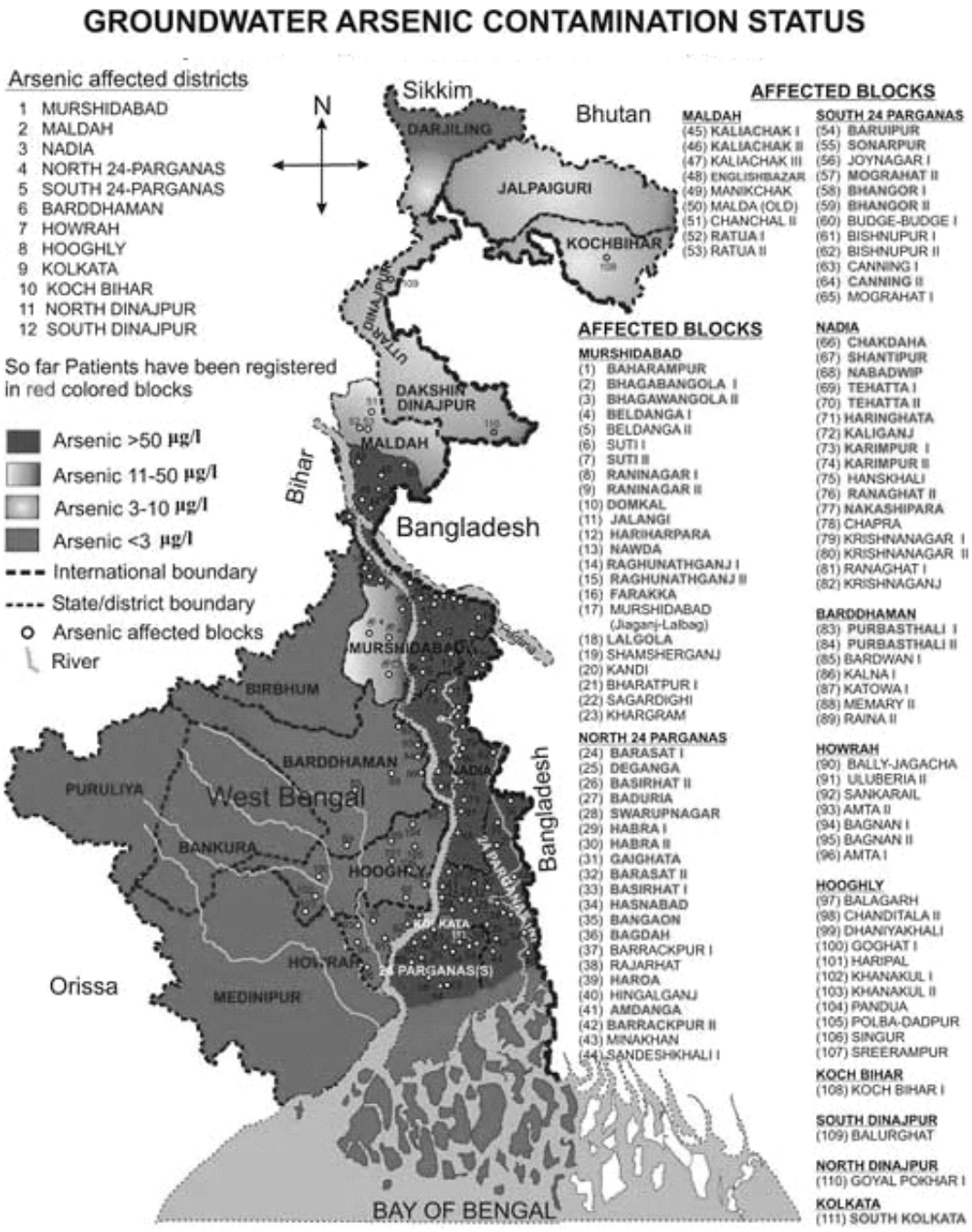

Figure 2.1 Arsenic affected area in West Bengal [Sarkar et al., 2016]

2.2 Different sources of Arsenic in water:

Majority of researchers agree that the main origin of arsenic in groundwater is geological relatively than others.

2.2.1 Geophysical source:

Theoretically it may be seemed that arsenic containing sulfide minerals (arsenopyrite) or their another products had been carried out in the geologic past from some earliest volcanic belt such as the foot hills of Himalayas and deposited with the alluvium in the delta part of Ganga-Brahamhaputra Basin which lies in West Bengal and Bangladesh. These buried arsenic minerals under the alluvium of Bengal delta are triggered to be responsible for water arsenic contamination [**Roy & Saha, 1999; Semedly & Kinniburgh, 2002**].

Excessive abstraction of groundwater facilitates to invade the environmental oxygen into the well which enhances the oxidation of arsenopyrite beneath the aquifer resulted in leaching the arsenic into water [**Das et al., 1996; Chowdhury et al., 1999; Chakraborti et al., 2004**]. Reductive suspension of ferric-oxyhydroxide contains precipitated arsenic that is responsible for aquifers contamination in West Bengal and another aquifers of alluvial [**Nickson et al., 1998; Nickson et al., 2000**]. Microbial reduction [**Chatterjee et al., 2004; Akai et al., 2004**] and carbon reduction [**Ashraf et al., 2002**], are recommended by various researchers. In corresponding to Saha et al. (**1997**) and Acharyya et al. (**2000**) the arsenic contamination in the groundwater of lower gangetic delta is enriched with arsenic riched residues by means of water transportation through water from the Chotonagpur-Rajmahal plateau and accumulated in sluggish flow in reduced form. Continuous over pumping elicited the reduction mechanism by encouraging

downward movement of underground water comprising massively falling degraded organic yields [**Acharyya, 2002**].

2.2.2 Geochemical resource or leaching of arsenic:

A recent research study as geo-chemical aspect continued extensive pumping of underground water for agriculture. The supply of excess oxygen is to be responsible for the hydrolysis of arseno-pyrite that generates toxic arsenic acid with ferric arsenite, while ferric arsenite turns into arsenious acid, which are considered as the pollutants of the ground water [**Roy & Saha, 1999; Acharyya, 2002**].

2.2.3 Organo-arsenic source:

Arsenosugar, arsenocholine, arsenobetaine tetra methyl arsonium salt and arsenic containing lipid, etc. are the main example of organo arsenic compounds which are abundantly found in marine bios as well as terrestrial species also. Organo arsenic is less harmful and mild toxicant than inorganic form of As^{3+} and As^{5+}. Organo arsenic is found in a variety of organisms for example plants, crabs fishes etc. Inorganic arsenic is converted as an organo-arsenic by the biomethylation process which easily incorporated in the biomass by the ingestion of arsenic contained food and aquifer. (**Mishra et al., 2017; Thompson, 1993; Caumitte et al., 2012**).

2.2.4 Pesticidal source:

The use of arsenical pesticides e.g. Monosodium methyl arsenate (MSMAsV), Disodium methyl arsenate (DSMAsV), Dimethyl arsinic acid (DMAsV) or cacodylic acid (CCA) causes a potential significant human health concern. These types of pesticides are used abundantly in agricultural purpose, pollutes the crops, surface soil and water also. The uses of inorganic arsenics as herbicides and

rodenticides cause the environmental recourses of arsenic. [**Chatterjee & Mukherjee, 1999; EPA, 2000**].

2.2.5 Industrial sources Arsenic:

a) Environmental contamination with arsenic, combustion of arsenic rich coal to generate power in power stations has been depicted from numerous countries, including India, Slovakia, China. Burning of local coal is generally contained about 900 – 1500 mg arsenic per kg coal. The uses of arsenic rich coals in thermal power plants of India are responsible for the arsenic pollution in air, soil and water [**Thornton & Farago, 1997].**

b) Copper melting factories where arsenic is obtained as a byproduct during the copper production. [**Mirza et al., 2014**].

c) In gold mining activity as continues the arsenic pollution because the coexistence of arsenic and gold bearing minerals. [**Straskraba & Moran, 1990**].

2.3 Human affection by arsenic:

Human can affect by arsenic in two ways such as occupational way and non-occupational way.

In non-occupational human exposure occurs principally via arsenic contaminated air, food and water. Contaminated water is the prime source of inorganoarsenic (both III and V) form. The highest values of arsenic is found in cereals, pulses, vegetables and fruits which are cultivated in contaminated soil and use of contaminated water as irrigation.

Although arsenic is present in food in various organical forms along with inorganic compounds, several lessons have stated about the total amount of arsenic in different

food and beverages only. Consistent with previous findings, amount of total arsenic is maximum in the marine foods samples ranging between 160 ng per gram in sweet water fish to 2360 ng per gram in salty water fish [**Sankar et al., 2006**].

Some studies noted that pulmonary exposure may include up to approximately 10µg/day in a smoke and about 1µg/day in a non smoker and more in polluted areas. The occupational human exposure is reported due to working in high arsenic concentration (>10µg/m^3) zone for several times [**Bencko, 1995**].

2.4 Absorption, metabolism, distribution and excretion of arsenic:

2.4.1 Absorption:

Arsenic is present in four forms as arsenate, arsenite, arsenic and arsinic. Inorganic arsenic is more intoxicant than organoarsenic which is found in trivalent and pentavalent state in nature. Both soluble forms of arsenic compounds are hurriedly and extensively absorbed from the GI tract (**Vahter & Concha, 2001**).

2.4.2 Metabolism:

After absorption of arsenic, biomethylation is occurred in liver by enzymatical and non enzymatical methylation process. It has been explored that the inorganic form of arsenic is detoxified by methylation and methylated by the attendance of methyl donor SAM (S-adenosyl methionine) along with glutathione (GSH) by help of arsenic methyltransferase (AS3MT) and resulted monomethylated [e.g., monomethylarsonous acid (MMA^{III}) monomethylarsonic acid (MMA^{V})] and dimethylated arsenical products [e.g., dimethylarsinous acid (DMA^{III}), dimethylarsinic acid (DMA^{V})], which readily eliminated by micturition [**Gamble, 2006; Faita et al., 2013].**

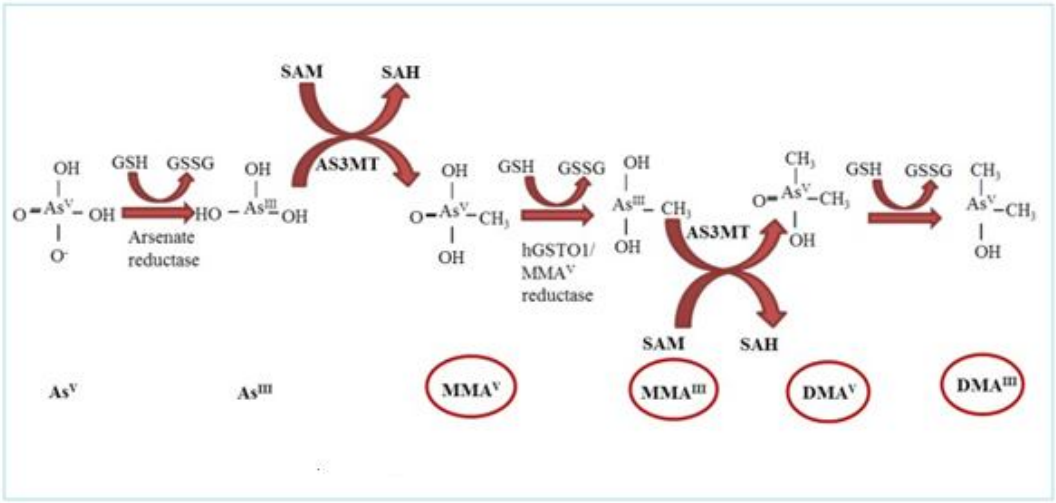

Figure 2.2: Schematic diagram of the pathway of inorganic arsenic metabolism in liver.

As^{5+} (Arsenate) is converted into AS^{3+} (arsenite) by the arsenate reductase concomitant GSH (glutathione) and GSSG (glutathione disulfide). The enzyme arsenic-3-methyltransferase (AS3MT) converts As3+ into MMA^{5+} (Pentavalent monomethyl arsonic acid), with S-adenosyl methione (SAM) acts as the methyl donor and, hydrolyzed into S-adenosyl homocysteine (SAH). Following the reduction of MMA^{5+} to MMA^{3+}, further methylation and reduction process DMA^{5+} is reduced into DMA^{3+} **[Faita et al., 2013].**

The final products of ingested arsenic are MMA and DMA which are readily eliminated from the body during micturition [**Faita et al., 2013; Gebel, 2002**]. DMA is capable to form ROS by reacting with molecular O_2 (oxygen) resulting in the formation arsenical metabolites such as dimethylarsenic radical and the dimethylarsenic peroxyl radical [**Tabacova et al., 1992; Yamanaka et al., 1990**]. Inorganic arsenic has been revealed to inhibit the numerous antioxidant systems in the body, such as glutathione, glutathione peroxidase, thioredoxin reductase, and superoxide dismutase. The accumulation of ROS, hydroxyl radicals, superoxide

radicals and H_2O_2 cause abnormal gene expression at lower concentrations and denaturation of proteins, lipids, and DNA in greater concentrations which ultimately progress to cellular death [**Maiti et al., 2012; Acharyya et al., 2015**].

2.4.3 Distribution:

The tissue distribution of arsenic in body accompanied with extent of ingestion and the type of arsenical compounds. Arsenic is deposited in liver, heart, kidneys and lungs abundantly whereas least amount found in muscular and nerve tissue. This metalloid is also expansively deposited in hair and nails due to profound thiol content (-SH) in keratin. The parity of chemical reactivity of arsenic with phosphorus, it is accumulated in bone and teeth also and retained there for extensive duration. Arsenic has also an ability to cross the placental barrier and consequently quantity of arsenic in umbilical cord is alike to the maternal blood. The frequent intake of aresinous oxide promotes the accumulation of arsenic notable concentration in the tissue of brain and also spinal cord [**Mazumder, 2005; Chattopadhyay & Ghosh, 2010**].

2.4.4 Excretion:

Arsenic is execrated from body through urine, feces, milk, sweat, hair, skin and lungs. Urinary route is the main route for excretion of arsenic from human. Dimethyl arsenic acid (DMA^{+5}) is the chief form of arsenic which eliminates via urine by the multistep methylation process of arsenical trivalent and pentavalent compounds. Half-life of arsenic is 72 - 120 hrs. According to the construction of organic arsenicals where As^{3+} or As^{5+} is covalently bonded with a carbon atom. The organic arsenicals are readily excreted hurriedly in respect of inorganic forms [**Gamble et al., 2006; Tabacova et al., 1992**].

2.5 Arsenic is in food, hairs, nails and body fluids:

Several relative studies showed that arsenic is emitted out from the blood quickly so, blood is not considered as a biomarker, particularly for low level exposures. Urinary arsenic is the critical marker of instant exposure of inorganic forms of arsenic and it is used as the chief indicator of exposure. Different studies reviled that after ingestion of arsenic about 60-75% elimination is occurred through the urine [**Hopenhayn-Rich et al., 1996**].

Previous studies expelled that the several zones of West Bengal the arsenic concentration in water is $4^{1}/_{2}$ to 5 times more than the tolerable limit i.e., 0.4 ppm suggested by WHO that arsenicosis can develops insidiously following six months to few years depending on the amount of arsenic ingestion and at some places it seems to 75 times [**Roy & Saha, 1999].** Usual arsenic content of creatinine in child values from $5^{1}/_{2}$ parts per million to 13 parts per million and it rises to 17 parts per million in children when both parents of children have smoking history [**Roy & Saha, 1999**]. However, arsenic content in creatinine of normal adults who do not regularly consume sea food is slightly more than 12.0 ppm whereas in Swedish people who consume large amount of sea fish, crabs etc. more than once a week, the arsenic content of creatinine is around 40.0 ppm [**Roy & Saha, 1999**]. Again, the mean arsenic level in human hair is 0.68 ppm in rural and 0.75 ppm in urban areas respectively but the hair of the Eskimos shows 5 times more arsenic than the normal level due to high intake of fish containing organo-arsenic [**Roy & Saha, 1999**]. In this perspective, it appears quite strange that an insignificantly small concentration of ionic arsenic like 0.05 to 0.06 ppm in drinking water can be in course of time

highly toxic to human health. Characteristic daily intake of arsenic from food for an U.S. adult is consumed at about 10-30 μg/day [**Chappell et al., 1997**].

2.6 Toxic effect of arsenic:

Arsenicosis may be developed insidiously after several months to few years depending on the quantity of arsenic intake. Human can expose to arsenic through drinking water or inhalation of arsenic dust particles. Though, there are medicinal values to combat against the numerous diseases, but recurrent uses pesticides, herbicides, insecticides, rodenticides, and food preservatives and as consequence use of arsenic contained fossil fuels are adequate to victimize in the aquatic environment in addition to human health [**Jomova et al., 2011**].

2.6.1 Toxic effect of arsenic on skin:

Arsenicosis mediated dermatological manifestation comprise melanosis, keratosis, hyperkeratosis and leucomelanosis Dermatitis has been frequently noted in chronic exposure of arsenic. The dermatological manifestation is hyperkeratinization on the skin (mainly on the palms and soles), formation of various hyperkeratinized warts or corns and skin hyperpigmentation along with hypopigmentation of spots. The classic appearance of the dark brown patch with scattered pale spots described as “rain drops on the dusty road” [**Chen et al., 2003**]. Leuko-melanosis and hyperkeratosis in the 2nd stage which eventually leads to skin cancer as Bowen's disease and cancer of squamous cell and basal cell by the methylation of cytosine base in DNA that promote the expression of oncogene or suppress the expression of tumour suppressor gene [**Rahman et al., 2009; Roy and Saha 2002].**

2.6.2 Arsenicosis on haematopoesis:

Several research studies noted on experimental animals arsenicosis disrupts the haematoblast cells causes inhibition of the erythropoesis rate resulted markedly diminution in erythrocyte counts, haemoglobin concentration (Hb%), haematocrit value (PCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV) [**Maiti et al., 2012**].

2.6.3 Effect of arsenic on Gastrointestinal (GI) system:

Accidentally arsenic injury related some preliminary symptoms with nausea, vomiting, abdominal ache and profuse watery diarrhoea. The extensive fluid loss results death due to massive emission from the GI tract including extreme dehydration, diminished blood volume in circulation and choking of circulation. In addition to postmortem investigation the reports are oesophagitis, gastritis, and hepatic steatosis also. Arsenic exposure significantly inactivate up to 200 enzymes, impaired with hepatic injury by the elevation of SGPT and SGOT activities. Reports revealed that arsenic mediated a severe necrotic and apoptotic hepatic injury could modulate the generation of liver functional markers **[Chattopadhyay et al., 2003; McVicker et al., 2009**]. Liver cirrhosis has been reported in smelter workers after chronic exposure to arsenicals. Moreover, arsenic was demonstrated to cause infiltration of inflammatory cells in the hepatic periportal areas [**Liu et al., 2002**]. Fatty infiltration in the hepatic tissue along with fibrotic changes has been informed distinctly in arsenic-exposed rodents [**Liu and Waalkes, 2008**]. Arsenic induced higher concentration of lipid along with its peroxidation might be responsible for oxidative damages in tissues [**Yang et al., 2007; Shila et al., 2005**]. The NPSH

level is direct determinant of GSH pool, decreased after arsenic exposure in the present study [**Mieyal et al, 2008; Forman et al, 2009**], especially those engaged in cellular energy pathways and DNA synthesis and repairing.

Arsenic toxicity impaired with glucose uptake and causes tupe-2 diabetes by the disruption of different thiol (-SH) containing enzymes in glycolytic pathway. Glycogenic and neoglucogenic pathways are inhibited due to acute arsenic poisoning [**Kulshrestha et al., 2014**]. Arsenic induction expressed the gene transcription factors might be correlate to diabetes threat. However, the impact of arsenite on IUF-1 and PPARγ were conflicting in diabetes progression [**Navas-Acien et al., 2006**]. The inorganic arsenic disrupts the Kerbs Cycle impaired with pyruvic acid metabolism [**Krebs, 1933**] and parity to phosphorus, interference the ATP production during oxidative phosphorilation also [**Kennedy & Lehninger, 1949**].

2.6.4 Effect of arsenic on neuron system:

Some researchers suggested that arsenic is a xenobiotic agent impaired with cognitive deficits memory, lower IQ and learning disability [**Tyler and Allan, 2014**]. In chronic exposure of inorganic arsenic reduced the glutathione (GSH) in cellular level the main antioxidant, causes the brain cell death. Gestational exposure to inorganic arsenic impairs NMDAR (N-methyl-D-aspartate) subunits expression in the hippocampus affecting spatial memory. In exposed populations, presence of arsenic in cord blood [**Concha et al., 1998; Hall et al., 2007**] indicates that arsenic can transfused into the fetus also a crucial agent which impairs to the neural tube defects of the developing embryo [**Shalat et al., 1996**].

2.6.5 Effect of arsenic on Immunology:

It is reported that arsenic is an immunomodulater causes the alterations of cellular and humoral immunity by producing irregular immunoglobulins along with a alteration of different immunochemical parameters (**Haque et al., 2017]** like as elevation of abnormal values of ceruloplasmine, than that of control subjects followed by a immunosuppressive effect through the formation of malignant tumor cells [**Kim et al., 2019**]. Moreover, arsenic exposed mice showed to inhibition of the primary antibody response [**Sikorski et al., 1991**), enhances mortality owing to bacterial infection [**Hatch et al., 1985**].

2.6.6 Arsenicosis and DNA:

Chronic ingestion of arsenic causes cancers of the many organs such as in liver, kidney, lungs, prostate, urinary bladder and skin etc. [**Brown & Chen, 1994**]. Study has been revealed that arsenic has no direct effect on with DNA [**Rossman, 2003**] due to poor mutagenecity [**Pierce et al., 2012**]. However, in spite of low capability of arsenic to generate mutations, it influences the mutagenicity along with other carcinogens. For example, the mutagenic activity is synergistically amplified by the application of arsenic in UV-exposed mammalian and human cells [**Li & Rossman, 1991; Lee et al., 1985**].

Studies informed that arsenite can bind with the -SH groups (sulfhydryl) of proteins and down regulates many biochemical reaction. Arsenate is a phosphate analogue which impedes the phosphorylation reactions. Several studies established that the free radicals are generated during cellular metabolism of arsenic. Oxidative stress has been associated with the expansion of arsenic allied diseases along with cancers. Moreover, ROS and also reactive nitrogen species (RNS) might be concern to

directly correlate in oxidative injury to proteins, lipids and DNA in arsenic exposed cells. Oxidative stress mediated over expression of P^{53} can induce c-fos mRNA and protein. Several studies highlighted that c-Fos regulated genes intended for tumorigenesis and leading to invasive proliferation of malignant cells. The level of C-fos mRNA magnitudes transiently in heat stress or Na-arsenite healing conditions which stimulate expression of hsp70 mRNA in cultured cell lines [**Ding et al., 2005; Mahner et al., 2008**].

Cytotoxicity and chromosomal aberrations are shown in limphocytes of some people exposed to arsenic, e.g. wine-growers during handling arsenic-containing pesticides. It is reported that the cultured cells in arsenic containing medium responsible for the mitotic arrestation and chromosomal damage [**Nordenson et al., 1981**].

Arsenic exposure is connected with base damage in dsDNA (double stranded) by the free radical generation [**Gomez et al., 2005**]. Furthermore, arsenic results the formation of apurinic-apyrimidine sites (AP sites) in DNA ultimately leads to gene mutation [**Martinez et al., 2011**]. Arsenic intoxication is allied to the expression of oncogene by inhibiting the tumor suppressor gene via DNA hypermethylation. [**Chanda et al., 2006].** Both the hypermethylation and hypomethylation of DNA in arsenic intoxicated state are mediated through modification of S-adenosyl methionine [**Chanda et al., 2006**]. The arsenite toxicity (As^{3+}) is determined by its binding to sulfur bearing protein molecules of the organ [**Zampella et al., 2012**]. Arsenic can develop DNA injury via genetic in addition to epigenetic pathways [**Salnikow and Zhitkovich, 2008**].

2.6.7 Effect of arsenicosis on Reproduction:

Literature review so far covered till the date explored that there is a very few data about the arsenical toxicity on reproductory parts. Some cross sectional studies noted that arsenicosis enhances preturmed delivery, unwanted abortion and also stillbirth. Inorganic arsenic mediated toxicity is responsible for fetal deformity, retardation of growth along with fetal morbidity [**Chattopadhyay et al., 2003**].

2.6.8 Toxic effect of arsenic on Male Reproduction:

There are very limited evidences to us about the effect of arsenicosis on human male reproductive system [**Saha et al., 1999; Kim & Kim, 2015**]. The arsenic mediated toxicity was first studies in mice, followed by in fishes [**Shukla and Pandey, 1984**]. In the investigational studies showed that there are drastically degenerative changes occurred in the seminiferous tubules decrease the diameter of interstitial cell of leydig according to administration of high concentration of arsenic. Orally administrated male mice to arsenic trioxide (As_2O_3) at the dose of 0.5 mg/kg for one month showed the higher concentration of cholesterol and significant inhibition of Δ^{5-}3β- HSD and 17β-HSD. The testis (male reproductive parts) is synthesised testosterone from cholesterol precursor [**Kabbaj et al., 2003**] where Δ^5, 3β-HSD generally converted pregnenolone to progesterone and 17β-HSD generated testosterone from androstenedione.

Some worker revealed that significantly attenuation of plasma concentration of FSH, LH and testosterone may impaired the alteration of pituitary-gonadal axis causes the disruption of prosteto-somatic and seminal vesiculo-somatic indices [**Sarkar, 1991; Pant et al., 2001**].

2.7 Reactive Oxygen Species and antioxidant properties:

2.7.1 Cellular machinery system of ROS (Reactive Oxygen Species) formation:

ROS are oxygen containing radicals (molecule or atom) that possessing one or more unpaired electrons [**Bae et al., 2011]**. The legend properties of free radicals are:

a) Unstable, high reactivity with short life (μs).

b) Autocatalytic and variegated chemical reactivity.

c) Yield *in vivo* and *in vitro* both.

Generation of free radicals may be in three ways:

a) By the single electron transfer.

$$A + e^- \longrightarrow A^{-\bullet}$$

b) By the homolytic fission.

$$X : Y \longrightarrow X^{\bullet} + Y^{\bullet}$$

c) By the Ion formation due to hydrolytic fission.

$$X : Y \longrightarrow X^- : + Y^+$$

The major free radicals are generated as the byproduct during the electron transport (in endoplasmic reticulum and in mitochondria), auto-oxidation of the some substances like Vitamin-E, Vitamin-C, GSH, selenium, thiols and adrenaline. Intoxicative substance such as arsenic can also responsible to generate free radicals [**Tabacova et al., 1992**].

2.7.2 Mechanism of free radicals generation are as followed:

In the first step super oxide radicals ($O_2^{\bullet -}$) are produced. Then, these harmful super oxide radicals are converted into H_2O_2 and O_2 by superoxide dismutase (SOD) in cell by dismutation reaction. Next, peroxisome's enzyme catalase and glutathione peroxidase converts H_2O_2 to form hydroxyl radicals ($OH^{\bullet}$) radicals. At the last step this $OH^{\bullet}$ may interact with the membrane phospholipids leads to the formation of peroxyl radical ($ROO^{\bullet}$), which can enter a chain reaction resulted formation of an alkoxyl radicals ($RO^{\bullet}$) and singlet oxygen ($^1O_2$) [**Phaniendra et al., 2015**].

2.7.3 Damaging reaction of free radicals:

Inspite of beneficial roles, free radicals are commonly toxic to cells, causes subsequent cellular damages such as lipid peroxidation, protein, cellular membrane and nucleic acid. Generally lipids are highly susceptible biomolecules. Cellular membrane contains polyunsaturated fatty acids (PUFAs) which are frequently affected by oxidizing radicals. This process is known as lipid peroxidation, which causes to increase the membrane rigidity, decrease the activity of sodium pumps and alter the membrane permeability and alteration of membrane receptor activity. Lipid peroxidation Impaired of coronary heart disease (CHD), adrenal hypertrophy, ageing, Female gonadal dysfunctions, cancer etc. [**Suresh et al., 1994**]. The schematic presentation of lipid peroxidation is as followed:

$$LH + R^{\bullet} \longrightarrow L^{\bullet} + RH$$

$$L^{\bullet} + O_2 \longrightarrow LOO^{\bullet}$$

$$LOO^{\bullet} + L\,H \longrightarrow LOOH + L^{\bullet}$$

$$LOOH \longrightarrow L^{\bullet}, LO^{\bullet}, LOO^{\bullet}, \text{aldehydes.}$$

Where, LH = Targeted PUFA

$R^{\bullet}$ = Initiating Oxidizing radical

$L^{\bullet}$ = Fatty acid radical

$LOO^{\bullet}$ = Fatty acid peroxyl radical

$LOOH^{\bullet}$ = Lipids hydroperoxides

2.7.4 Arsenic and free radicals:

Arsenic is a well-characterized inducer of oxidative stress. For example, a natural fish oil named squalene, is a potential agent in the attenuation of $NaAsO_2$-madiated ovarian sister chromatid exchange and generation of ovarian micronuclei in Chinese hamstar [**Fan et al., 1996].** Catalase and superoxide dismutase (SOD) were feasible to reduce the arsenic mediated sister chromatid exchanges tendency in peripheral lymphocytes of individual. [**Maki et al., 1998**]. This metalloid acts as inducer of oxidative stress in human fibroblasts *in vitro* [**Yih et al., 2002**] and in mice lung in vivo [**Wei et al., 2018**]. These results point out that the oxygen radicals may involve in the toxicity by arsenic induction.

2.7.5 Role of antioxidants against on free radicals: [Poljsak and Fink, 2014]

Arsenic mediated oxidative stress is detoxified by the innate protection machinery of antioxidant that acts free radicals scavengers. In cells, there are several enzymatical and non-enzymatical antioxidant system evolved to play the detoxification.

The substrate of some enzymatic and non-enzymatic antioxidants is as follows:

Antioxidant Name (Enzymatic)	**Substrate Name** (Detoxification)
i) Catalase	---------- H_2O_2
ii) Glutathione peroxidase	---------- H_2O_2
iii) Peroxidase	---------- H_2O_2
iv) SOD (super oxide dismutase)	---------- superoxides

Antioxidant (Nonenzymatic)		**Substrate Name**
i) Vitamin-C	----------	Scavenges H_2O_2, OH
ii) Vitamin-E	----------	Scavenges H_2O_2, OH
iii) Transferrin	----------	Scavenges H_2O_2, OH
iv) βcarotine	----------	Protect against lipid peroxidation
v) Selenium	----------	Traps heavy metals and metalloid
vi) GSH	----------	Reduces free radicals

2.7.6 Role of antioxidant on arsenic toxicity and female reproduction:

Few studies have been revealed regarding the female reproductivity and role of antioxidants on arsenic toxicity. There are some reliable markers impaired of *in vivo* oxidative damage has recently been established and among them, peroxidase is one of the prime enzymatic biomarkers causes oxidative damages [**Chaterjee et al.,**

2003, Phaniendra et al., 2015]. Not only that, peroxidase has been associated with estrogen action in the uterus but also impaired with uterine receptivity [**Baiza-Gutman et al., 2000**]. Moreover, elevation of catalase, glutathione reductase activities are noticed in corpus lutea of pregnant pigs, which suggest that diminution of ROS, is associated with high functional activity of antioxidant system [**Eliasson et al., 1999**].

After administration of sodium arsenite a remarkable diminution is found in ovarian and uterine mass, ovarian Δ^5-3βHSD and 17-βHSD enzyme activities in rat model. That suggested low plasma level of estradiol which disrupt the Hypothalamico-hypophyseal-gonadal axis resulted low plasma FSH and LH which responsible for retardation of follicular growth. Co-treatment of L-ascorbic acid along with arsenic in pregnant Wistar's rat has been reported that quantity of arsenic in placenta was intensely drops in compared with arsenic treated group. Studies also reported that arsenic toxicity in ovary and uterus are protected significantly by supplementation of ascorbic acid **[Chattopadhyay et al., 2001**].

Arsenic, the chief water pollutant pretends its carcinogenic and genotoxic activities generally in the type of hyperkeratosis, gangrene, and skin cancer as well as tumoural growth in alveolus, liver, adrenal gland and ovary also. [**Rahman et al., 2009; Roy and Saha, 2002**]. Severe diabetic disorder and reproductive abnormalities have also been reported as a causal effect of arsenic contamination [**Rahaman et al., 2009; Pott et al., 2001]**. Our recent finding depicted that exhaustion of antioxidant machinery system and elevation of DNA fragmentation in arsenic induced human population resulting the tissue necrosis and carcinogenesis in integument and other organs [**Maiti et al., 2012**]. After ingestion, arsenic is

hurriedly metabolised and converted into monomethylated, dimethylated and trimethylated species through the biotransformation procedure. Metabolism of arsenical compounds is correlated to the assemble of free radicals and ROS which eventually encourages oxidative stress by a significant drop down of reduced glutathione and enhance the DNA breakage [**Maiti and Chatterjee, 2001; Yamanaka et al., 1990**]. Certain drugs such as BAL, DMSA are prescribed to combat against arsenicosis as the chelating agents, but these have several adverse outcome resembling nausea, abdominal pain, etching, hypertension [**Inns et al., 1990**]. Some previous studies expressed that ascorbic acid, tocopherol and selenium [**Mahata et al., 2008]** are antagonist and used as nutritional supplements against arsenic toxicity [**Chattopadhyay et al., 2001]**. Moreover, supplementation of HCG (human chorionic gonadotropin) is restricted the alteration of arsenic mediated utero-ovrian steroidogenesis via hypophyseal-gonadal axis along with hypophyseal-adrenal axis [**Chattopadhyay & Ghosh, 2010**]. Herbal remediation of arsenic-induced hepatic toxicity by the extracts of *Moringa olifera* seed has also been depicted [**Chattopadhyay et al., 2011**]. Various other herbal product like quercetin, curcumin, resveratrol, mono-isoamyl dimercaptosuccinic acid and some vital phytochemicals have been established as antagonist to arsenic-madiated oxidative stress, DNA damages, liver tissues deformities, fibrotic and carcinogenic changes [**Ghosh et al., 2010; Mishra et al., 2009; Maiti et al., 2014]**. The lipid soluble Vitamin-E is the least toxic than the others vitamins family [**Gerald & Combs, 1992**], plays a much better pivotal role to reduces oxidative damage to lipids and bio-membranes *in vivo* in comparison with Vitamin-C or β-carotene [**McCall & Balz, 1999**]. Vitamin-E chain-breaking capacity is not only defense against peroxidative reactions but it renovates the membrane integrity also [**Lucy, 1972**]. It

is strongly evident that α-tocopherol could be defend the cellular membrane integrity against peroxidation by the diminution phospholipase A_2 [**Douglas et al., 1986**] followed by attenuation the augmentation of unesterified fatty acid which look like to mainly vulnerable to peroxidative injury. Some evidence revealed that Vitamin E scavenges enzyme and protected the ovarian and uterine cells by combating against the arsenic mediated ROS [**Chattopadhyay et al., 2001**].

Nowadays some researchers have shown Selenium intended to detoxify the arsenic from the alive cells. Nutritional supplementation of sodium selenite is efficient in significant reduction of the arsenic mediated mutagenic and genotoxic effects [**Biswas et al., 1999**; **Beckman & Nordenson, 1986**]. A lower defensive activity of selenium was observed against arsenic mediated toxicity in suspension culture of mice fibroblast LA115 [**Rossner et al., 1977**]. Co-administration of selenium and gallium arsenide recommended that selenium has some preventive roles in prohibition of gallium arsenide induced toxic effects by restoring the diminution of circulatory δ-amino levulinic acid dehydratase along with hepatic MDA production and instantly blocking the deposition of arsenic and gallium in tissue level [**Flora et al., 1999**]. Dietary selenium prevents the arsenic attributed abnormalities in reproductive system of female rodent might be change the hypothalamic–pituitary-ovarian axis by the stimulating adrenergic neurons and reduction of the oxidative stress by enhancing scavenging activities resulted natural occurring ovarian folliculogenesis [**Chattopadhyay et al., 2003**].

Reduced glutathione (GSH) acts as a non-enzymatic antioxidant [**Shila et al., 2005**]. According to evidents, GSH has been played as a protectant against oxidative injury of male and female reproductive units (gametes) [**Luberda, 2005**]. The exogenous

GSH enhances biliary elimination of arsenate (As^{+5}) by the creation of arsenic–GSH conjugates [**Gregus & Gyurasics, 2000; Csanaky & Gregus, 2005**], which might be play a crucial role in arsenic detoxification in vital cells. In vitro studies, the exogenous GSH ameliorates the activity of 17-βHSD in lead and cadmium intoxicated ovarian granulosa cell [**Priya et al., 2004**].

Since the female population from wide arsenic affected zone is suffering from infertility as a consequence of consuming arsenic contaminated drinking water [**Balabanic et al., 2011**], here we have taken an effort to justify whether vitamin B_{12} and folic acid are able to renovation of attenuate uterine disorders induced by this endocrine disruptor.

2.8 Wistar albino rats and Estrous cycle of this strain:

2.8.1 Estrous cycle in Wistar rat:

The Wistar albino strain was called laboratory rat Residence of it was at Wistar institute, Philadelphia in USA. It is a prolific type of animal and now reared in different research laboratories all over the world. It is relatively high resistance to infections and has a little frequency of fascicle growth of tumor. Lifetime of this animal is about 2-3 yrs. The reproductive maturity of this variety is at 6½ – 7½ weeks. Generally this female strain starts it's 1^{st} estrous at the age of 6 to 7 weeks. The estrous cycle of this variant persist usually for 4 to 5 days and during cycle a remarkable histological alteration is occurred in vaginal and uterine cells.

Estrous cycle is observed by showing the 'vaginal smear', a type of changes of vagina cells. This cycle does not happen during in pregnancy, pseudopregnancy or in lactating [**Young et al., 1941; Reeman, 1988; Long & Evans, 1922**].

2.8.2 Phases of the estrous cycle:

There are four phases estrous cycle noticed in this Wistar rat, according to cell types in vaginal smear and these are as followed:

Proestrus:

i) It is the 1st phase of estrus and lasting for 12 hrs in this Wister strain.
ii) Follicular enlargement.
iii) Vaginal smear shows the nucleated epithelial cells.
iv) High peak of estrogen levels
v) At the end of this phase female show the signs of acceptance of male.

[**Gonzalez, 2016**]

Estrus:

i) It is 2nd phase of estrus and characterized by the period of sexual receptivity when male is welcomed by the female.
ii) Duration in Wistar strain is 12 hrs.
iii) Each ovary in this phase generally contains 5-6 fully formed graafian follicles and medium sized follicles.
iv) Estrogen level decreases and LH surge occurs resulted ovulation is also noted.
v) At the last part of this phase many ruptured follicles are also noted in ovary.
vi) Proliferation of vaginal epithelial cells at basal surface is maximum and show several cell layers at the luminal surface that are deprived from blood flow.
vii) The vaginal smear shows cornified epithelial cells.

Metestrus:

i) This is the 3rd phase of estrus having the duration of 21 hrs.

ii) At this phase female has no capacity to accept male.

iii) Antral follicles are absent or very minimal in metestrus [**Parshad, 1989**] and corpora lutea are noted in ovary.

iv) The synthesis of progesterone in this phase reaches maximum level.

v) At this phase of vaginal smear show leukocytes and cornified epithelial cells.

Diestrus:

i) This is the 4th and last phase of estrus having the duration of 57 hrs.

ii) Degenerative corpora lutea are found in ovary and decreased the blood level of progesterone.

iii) As a result, the coiled blood vessels in uterus and vagina become fragile.

iv) This is the inactive phase of estrous cycle from the angle of mating, conception etc.

v) In this phase of vaginal smear shows leukocytes only.

2.8.3 Hormonal regulation of different phases of estrous cycle: [Hillier et al., 1980]

Proestrous phase:

According to follicular maturation following features are observed:

i) Granulosa cells proliferated in numbers.

ii) Layers of theca cells grow and encircled the follicle - theca cells differentiate into theca externa and interna.

iii) FSH provokes theca interna to synthesis testosterone from cholesterol.

iv) LH provokes Granulosa to synthesis estrogen from testosterone.

v) FSH is mainly responsible for growth of the follicles.

vi) Estrogen level is the highest during the late proestrus phase and does not increase rapidly until progesterone levels have begun to decline rapidly.

Estrous phase:

Due to the local effect of estrogen on the central nervous system following features are noted in this phase:

i) Estrogen exerts a positive feedback on the hypothalamic- hypophyseal axis resulting the stimulation of adrenergic neurons to secretion of GnRH.

ii) The release of peak levels GnRH leading to secretion of LH and FSH from anterior pituitary.

iii) At estrus, FSH rarely increases more than 2 times whereas LH increases 200-300 times.

Metestrous phase:

i) In this phase, generally female do not receive male and corpus lutium are developed in ovary. Following features are noted in this phase.

ii) Progesterone is secreted from the corpora lutea.

iii) High level of blood progesterone, causes endometrium proliferation along with inhibition in uterine contraction.

iv) PRL maintained the formed of corpus luteum and luteal progesterone controls the uterine and vaginal behavior.

Diestrous phase:

In this phase there is inovulation of corpus luteum in ovary.

Following event is noted in this phase:

i) Due to involution of corpus luteum blood level of estrogen and progesterone is below normal.

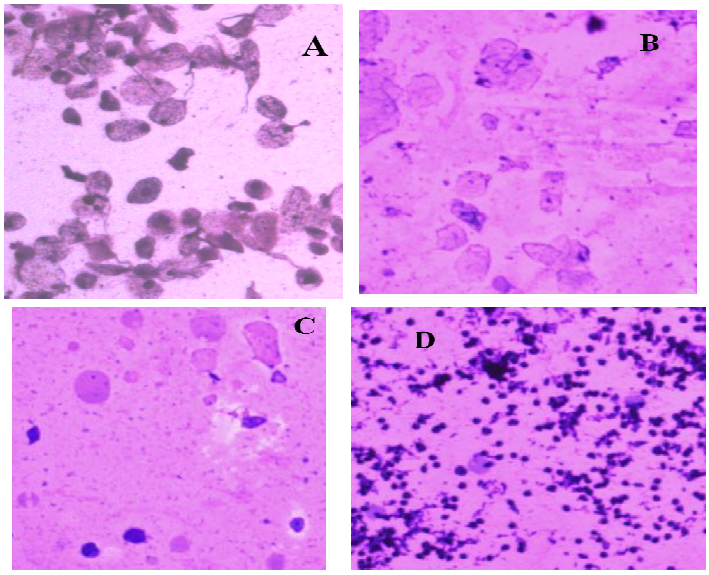

Figure 2.3: Phases of Wistar Rat's Estrous Cycle A: Proestrous B: Estrous C: Metestrous D: Diestrous

2.9 Ovarian Steroidogenesis:

Ovary is a steroidogenic organ, is responsible to synthesize estrogen and progesterone. Estrogens are three types i.e., estron (E1) estradiol (E2), estriol (E3).

In mammal, cholesterol is used as precursor for ovarian steroidogenesis derived from plasma LDL (low-density lipoprotein) and HDL (high density lipoprotein) [**Senger, 2005; Strauss et al., 1981**]. The HDL receptors and LDL receptors are present on the thecal membrane or corpus luteual membrane that bind and uptake the circulating HDL and LDL [**Hu et al., 2000**]. These lipoproteins are transported

to inner mitochondrial membrane by the help of cAMP protein [**Clark et al 1995; Andersen & Ezcurra, 2014**]. Ovarian steroidogenesis is a complicated process as specific cells of ovary are intended for specific steroid synthesis. Ovarian steroidogenesis is occurred in mitochondrial process followed by microsomal process. At first C27 cholesterol is converted into C21 pegnelelone by enzymatic cleavage of a 6C side chain of the C27 cholesterol, a rate limiting catalytic reaction by cyt-P_{450} side-chain cleavage enzyme present in [**Montano et al., 2009 ; Zachow et al., 2000**] mitochondria. This pregnanolone is converted into androstenedione in microsomal process followed the Δ^5 and Δ^4 pathway. In Δ^5 pathway by hydroxylation process, pregnalelone is converted into 17-hydoxypregnanelone in presence of hydroxylase. This 17-hydroxypregnanolone removes its acetyl group and transformed into DHEA (dehydroepiandrosterone) the precursor of androstenedione by the influence of lyase enzyme. Overall this mechanism is influenced by theca cells of ovary. On the other hand, in Δ^4 pathways androstenedione is produced from pregnenolone with intermediates 17-hydroxyprogesterone and progesterone by the enzyme complex (Δ^5,3β-HSD, 17α-hydroxylase and $C_{17\text{-}20}$ lyase) in presence of NADPH and molecular oxygen. This mechanism is operated by corpus luteum or granulosa cells. Δ^5,3β-hydroxysteroid dehydrogenase (Δ^5,3β-HSD) is responsible for conversion of DHEA into androstenedione (AD). This AD is rapidly converted into testosterone (T) by 17β-HSD activity and an aromatization process this T is converted estrone as estradiol intermediate [**Senger, 2005; Vanderhyden & Tonary, 1995].** The enzyme aromatase is involved for conversion of androgen to estrogen which is found in a

granular endoplasmic reticulum of granulosa lutine cells [**Gore-Langton & Armstrong 1994; Trembly et al., 1991**].

2.10 Ovarian folliculogenesis: A brief review :

2.10.1 Histological structure of different generation of follicles in folliculogenesis:

Folliculogenesis is a process in which some specific somatic cells recruited primordial folicle converts into graafian follicle contains ovulate its egg. Folliculogenesis is a continuous and has several stages in follicular development by the help of GnRH, FSH, LH and estradiol regulation. There are four type follicles like *Primordial, Primary, Secondary, Tertiary* and *Graafian follicles* [**Yong et al., 1922a & 1922b**].

Primordial Follicle: This follicle contains immature oocyte in dictyate stage bordered by one layer flat, squamous granulosa cells and a basal lamina. It is generally dormant, viewing sluggish to no biological activity. The process of initial recruitment the primordial follicle cells stimulation is regulated by different stimulatory and inhibitory hormones and locally formed growth factors. This theca is cytodifferentiate into theca interna and theca externa

Primary follicle: Primary follicle's oocyte is bordered with single or more layer cuboidal granulosa cells. The oocyte genome becomes active and ready to transcription. Research shows that during folliculogenesis granulosa cells express FSH receptor by paracrine or autocrine signaling pathway. Therefore oocyte growth along with the follicle diameter enlarges noticeably.

A glycoprotein named zonapellucida proteins make outer layer around the oocyte by polymerization called zona pellucida which separate the oocyte from the granulosa cells This zona pellucida contains species specific sperm binding molecules to facilate sperm attachment and penetration.

Secondary follicle: In secondary follicles cuboidal follicular epithelium of primary folicle is transformed into a stratified epithelium. Stoma-like theca cells acquisite around the basal lamina. Perplexing blood vessels are appearing in between theca layers for blood circulation to and from the secondary follicle. Thus secondary follicles becomes a multilayer structure contain an oocyte bounded by zona-pellucida, several cells layers (6-9) of granulosa, basal lamina, theca interna, blood vessels and theca externa.

Tertiary follicle: Multilayer secondary follicle transformed into vesicular follicles with the development of fluid-filled intracellular region, designated as antrum. The antral follicle along with cavity is nominated as tertiary follicles.

Graafian follicle: This tertiary follicle on preovulatory growth is known as graaffian follicle [**Freeman, 1994**]. The oocyte is attached granulosa cells and become eccentric, forming a hillock designated cumulus oospores [**Mossman & Duke, 1973; Chaffin & Vandevoort, 2013**]. The oocyte is surrounded granulosa cells with configuration a ring like arrangement known as corona radiata. The fluid recites in antrum is called liquor.

2.10.2 Mitotic and meiotic cell division of oogonium at different steps of folliculogenesis:

In the adult, the final maturation step at the oocyte begins within the follicle followed by the meiotic-I division. After 1st meiotic division, the oocyte is

transformed into secondary oocyte along with a polar body. This secondary oocyte is situated in tertiary or graafian follicle. After the first polar body extruded, ovulation occurs. This ruptured follicle discharged the secondary oocyte into the fallopian tube, started its 2nd meiotic division after fertilization by sperm [**Freeman 1994; Guraya, 1985**]. Therefore a short of duration of meiotic-II (2nd meiosis) arrestation or resting state is happening from ovulation and extended up to fertilization. After fertilization by sperm pronuclei, the secondary oocytes complete 2nd meiosis and forms mature oocyte and 2nd polar body.

2.10.3 Histological structure of atretic follicles:

Follicular atresia stars by degeneration or disorganization of oocyte Atresia of follicles in small preantral follicles [**Sahu, 1984; Kaur & Guraya, 1983**]. In atretic follicle, the granulosa cells are persist where as their oocytes are collapsed or faded away entirely. The granulosa cells of atretic follicles build up a marked activity of two hydrolytic enzymes, which forms are lysosomal enzymes like in nature. (**Parshad & Guraya, 1983**).

2.10.4 Hormonal regulation to maintain estrous cycle:

The initiation and regulation of estrous cycle in rodent is controlled by hypothalamico-hypophysial-gonadal (HHG) axis. Though folliculogesis is started independently in ovary but later its maintained by FSH, LH and estradiol. The following steps are:

1. After diestrus phase FSH an LH secretion is increased from adenohypophysis. FSH gives initiation to zonapellucida for expression of LH receptor by granulosa cells. LH introduces the synthesis and secretion of androstenedione and testosterone by the theca interna. Then this testosterone

is aromatised into estradiol by the granulosa cells in ovary. Pituitarian gonadodropins consequently operate follicular development and secretion of estradiol throughout the proestrus. This estradiol is essential for proliferation and differentiation of ovarian and uterine tissue.

2. Pituitarian gonadotrophin secretion is controlled by the hypothalamico GnRH (gonadotrophin releasing hormone). This GnRH secreted from hypothalamus by rhythmic pulses and come to the anterior pituitary via the circulatory system.

3. After increasing the estradiol level in plasma it gives a negative feedback on hypothalamus, resulted low secretion of FSH. Inhibin in granulosa cells also made a negative feedback to hypophysis that resulted low FSH secretion.

   Estradiol level rises in the morning, it riches in peak at around noontime and suddenly go down in the afternoon during day time in proestrus phase. At this preovulatory stage follicular estrogen exerts a positive feedback for LH discharge from pituitary along with consequent diminution in FSH emission [**Hillier, 1999**]. In that situation of development granulosa cells express LH receptor [**Hsueh et al., 1984**] located on thecal cells and LH surge is occurred that resulted ovulation and corpus luteum formation [**Hillier, 2001**].

4. Progesterone level rises in higher concentration during proestrus and has shown in peak during ovulation. During ovulation progesterone synergies with estrogen and give a negative feedback to hypophyseal gonadotroph cell resulted low secretion of gonadotrophin. Simultaneously peak of progesterone also gives a positive feedback to the hypothalamus for initiation

and secretion of GnRH which stimulates gonadotrophin to emission LH and enhances the LH surge.

5. After ovulation ovary is converted into corpus luteum (CL) by a complex hormonal controlling process. The CL secrets progesterone about 2 days before becoming non functional and degenerating over the course of several subsequent oestrus cycles [**Richards & Midgley, 1976**].

6. Luteal phase is maintained by the balance of luteogenic and luteolytic factor [**Hillier, 1999; Rothchild & Schwartz, 1965**]. Luteal steroid (progesterone) secretion is regulated by LH and paracrine signalling. The luteolytic factors impade the persistance of corpus luteum which controlled by LH also. In absence of HCG the lutolysis is progress.

2.11 An overview of vitamin B_{12} and folic acid:

2.11.1 Vitamin B_{12}:

Vitamin B_{12} is wellknown vital metalo-carbonical biomolecule. So, it is an organometallic, water soluble, odourless, tasteless, dark red crystalline compound. It is stable in normal pH but not alkaline pH.

Molecular Formula: $C_{63}H_{88}CoN_{14}O_{14}P$

Molecular Weight: 1355.388 g/mol

Chemical Names: Vitamin B_{12}; Cyanocobalamin.

IUPAC Name:

cobalt(3+);[(2R,3S,4R,5S)-5-(5,6-dimethylbenzimidazol-1-yl)-4-hydroxy-2-(hydroxymethyl)oxolan-3-yl] [(2R)-1-[3-[(1R,2R,3R,5Z,7S,10Z,12S,13S,15Z,17S,18S,19R)-2,13,18-tris(2-amino-2-oxoethyl)-7,12,17-tris(3-amino-3-

oxopropyl)-3,5,8,8,13,15,18,19-octamethyl-2,7,12,17-tetrahydro-1H-corrin-24-id-3-yl]propanoylamino]propan-2-yl] phosphate;cyanide.

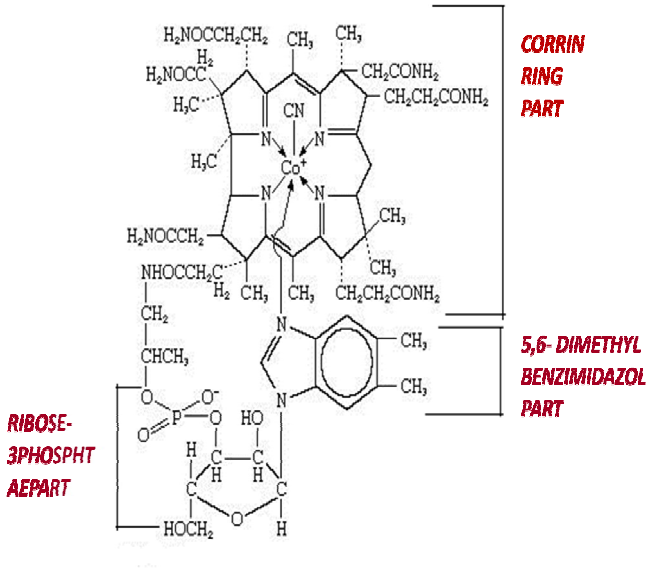

Figure 2.4 : Vitamin B_{12}

The complex structure of B_{12} bears a corrin ring with close proximity of porphyrin ring and is having central cobalt ion. Four coordination sites are occupied by the corrin ring out of six coordination sites. Fifth site contains dimethylbenzimidazole group. The variable sixth coordination site bears a cyano group (-CN), a hydroxyl group (-OH), a methyl group (-CH3) or a 5'-deoxyadenosyl group, respectively, to yield the four B_{12} forms. The hydrogenases and necessary enzymes related with cobalt utilization, involve metal-carbon bonds [**Jaouen, 2006**].

Fish, meat, egg, dairy products fortified cereals etc are rich in Vitamin B_{12}.

2.11.2 Folic acid:

Folic acid collectively known as Vitamin-B_9 which is in water soluble.

IUPAC ID: (2S)-2-[(4-{[(2-amino-4-hydroxypteridin-6-yl) methyl] amino} phenyl) formamido] pentanedioic acid

Formula: $C_{19}H_{19}N_7O_6$

Molecular Weight: 441.404 g/mol

Here six membered long chain molecules are having with single aryl ring that further attached dihydropteridine ring.. Folic acid is an odourless orange-yellow needles or platelets.

Leafy vegetables like spinach, asparagus, lettuces, beans, peas, fortified cereal products, sunflower, yeast, liver and liver products, and kidney are rich sources of folic acid.

2.11.3 Absorption and distribution of Vitamin B_{12}:

Using an intrinsic factor methyl-B_{12} is absorbed in the intestinal lumen by a diffusion process with an absorption rate of approximately 1% of the total ingested dose. According to human physiology of Vit-B_{12} the complexity and trend to misshaping encourages the vitamin B_{12} deficiency. Both the stomach and small intestinal digestive proteases contribute in the release of protein-bound vitamin B_{12}. Since acidic environment of gut liberates the vitamin from foodstuffs; therefore antacid and H^+ pump sluggish the absorption of B_{12}. This is the probable reason why elderly people suffer from B_{12} deficiencies due to less stomach acid [**Neale, 1990**]. Less-soluble, non-chewable supplement pill of B_{12} if ingested it may bypass the mouth and stomach and not mix with HCl in gastric juice, but acids are not

compulsory for the assimilation of free Vit-B_{12} unbound to protein; acid is needed only to restore naturally-occurring Vit- B_{12} from foodstuffs [**Seetharam & Alpers, 1982].**

B_{12} binding protein named haptocorrin or cobalophilin or R-protein is synthesized in salivary glands. When B_{12} is released from proteins in food by stomach R-protein binds with this vitamin [**Neale, 1990; O Proinsias et al., 2016]**. The IF (intrinsic factor) is the next another binding protein for Vit-B_{12}. This IF is produced by parietal cells of stomach by the influence of gastrin, pentagastrin and histamine in addition to the existence of food. In duodenum digestion of R-proteins release bound B_{12} by the pancreatic proteases which further combines with IF and constructs IF-B_{12} complex. B_{12}-IF complex is required for proper absorption of Vit-B_{12} and B_{12}-IF complex receptors are found on the terminal ileum's enterocytes of the small intestine. IF also shields the vitamins away to be catabolized by intestinal bacteria.

B_{12} is transported into the portal circulation following the recognition of IF-B_{12} complex by special ileal receptors. The vitamin is then transferred to a plasma transporter named as transcobalamin II (TC-II-B_{12}). However, in some events with normal blood Vit-B_{12} intensity [**Seetharam & Alpers, 1982]**. Subsequently lysosomal degradation of transcobalamin-II occurs and ultimately free B_{12} is released into the cytosol [**Seetharam & Alpers, 1982**]. Hereditary deficiency of the TC-II and their receptors might develop functional deficits of Vit-B_{12} and juvenile megaloblastic anaemia and B_{12} related abnormal biochemistry.

2.11.4 Folic Acid absorption:

To maintain the folate status in the body, regular uptake and proper absorption of folic acid is required. Dietary folic acid absorption is occurred by means of lessening

absorption gradients from jejunum to colon. Dietary folic acid exists as polyglutamets which transformed into folate monoglutamates by the enzyme folate reductase which present in jejunum mucosa of small intestine. The folic acid uptaking is pH dependent with an optimum at pH 5.5 (acidic). It is followed the Michaelis-Menten the enzyme kinetics pathway by a carrier-mediated transporter named Proton-coupled folate transporter (PCFT). A human proton-coupled, high-affinity folate transporter (PCFT) was invented and it was expressed that the mutated gene loss its function and could impaired for autosomal recessive hereditary folate malabsorption The effect of various organic molecules, ions and drugs on proton-coupled folate transporter-arbitrated folate transportation is depicted [**Visentin et al., 2014**]. After few years this machinery was again elucidated by the invention of the RFC (human reduced folate carrier) in the mucosa of colon. The intestinal mucosal RFC which is found in the apical brush-border of membrane was considered as a folate transporter also. The body can store about 20-70 mg of folates in the liver. After absorption folic acid is turned into dihydrofolate in the hepatic tissue by the influence of dihydrofolate synthetase and into tetrahydrofolate (FH4) by the enzyme dihydrofolate reductase. FH4 is transformed into 5, 10-methyleneFH4 by the help of serine hydroxymethyl transferase. FH4 along with its methylated species play a vital role as methyl donors.

2.11.5 Functional aspects of Vitamin B_{12} and folic acid:

Vit-B_{12} helps in the synthesis of RBC red blood cells and increases spermatozoids in semen [**Watanab et al., 2003**]. Loss of libido, incontinence, cervical dysplasia, infertility, menoorhagia, dysfunctional uterine bleeding is occurred in vit-B_{12} deficiency. The deficiency of Vit-B_{12} diminishes the folate metabolism resulted in

pernicious anaemia and megaloblastic anaemia. The assimilation of vitamin B_{12} is influenced by the intrinsic factor (IF). Either deficiency of IF or poor absorption by other reasons of vitamin B_{12} impair with pernicious anaemia (PA). This PA is an autoimmune disease which impairs the gastric mucosal injury, resulted the gastric atrophy. This evolves to destruction of the stomach parietal cells leads to deficit of gastric acid secretion (achlorhydria) and impedes intrinsic factor production, resultant the Vit-B_{12} malabsorption. The uncontrolled pernicious anaemia elevates the vitamin B_{12} deficiency, ultimately enhancing the megaloblastic anaemia and neurological disorders [**Andres and Serraj, 2012**].

Vitamin B_{12} plays a vital role in the methionine synthase reaction during formation of methionine from homocysteine and methyl-FH4 to FH4. According to 'methyl-folate trap' hypothesis failing of demethylation of methyl-FH4 with subsequent insufficiency of folate co-enzymes which obtained from FH4 is the critical lesion occurred due to vitamin B_{12} deficiency. Without coenzyme B_{12}, FH4 cannot be rejuvenated from its inactive storage form, 5-methyl FH4 leading to functional folate insufficiency resulted megaloblastic anaemia. Thus, Vit-B_{12} plays a crucial role in folate metabolism **[Fenech et al., 1997**].

Folate is essential for the generation and preservation of young cells. It is mainly essential during rapid cell division and development in childhood and gestation period. Both children and adults require folic acid to produce normal RBC and WBC (white blood corpuscles) and preventing anaemia. Scarcity of folic acid during pregnancy is response for NTDs (Neural Tube Defects); therefore, WHO recommended for intaking the folic acid mandatory during gestation period [**Shaw et al., 1995]**.

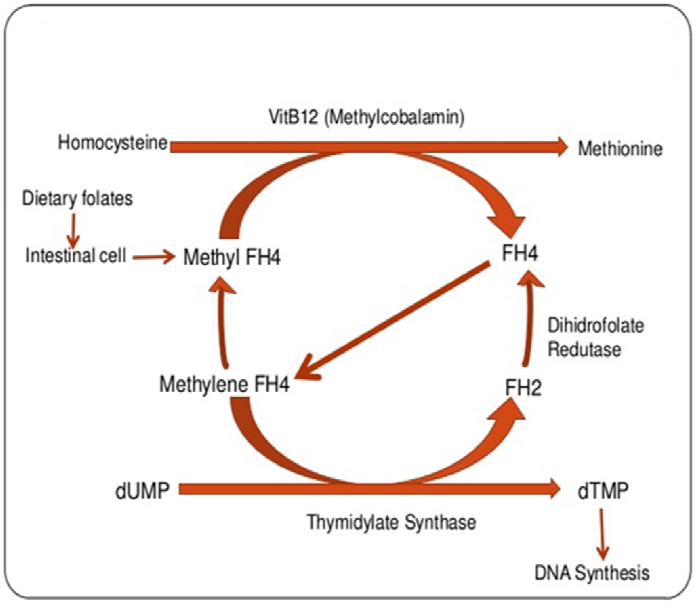

Figure 2.5: Role of Vitamin B_{12} and Folic acid in DNA Synthesis

Folic acid and Vit-B_{12} play a vital role in the preclusion of chromosomal breakage and prohibition the DNA hypomethylation. This action is negotiated during Vit-B_{12} concentration is reduced due to diminish the activity of methionine synthase, lesser quantity of SAM might be decrease the DNA methylation which tern to folate become deficit for the transformation of dUMP (deoxyuridine monophosphate) to dTMP (deoxythymidine). The most elucidation about the chromosomal breaking that the low level of folic acid is impaired to extreme uracil misincorporation into DNA which creates a mutagenic lesion and induces DNA strand breaking during repairing.