Chapter 10

Review of Literature
Review of literature

Premature ovarian failure is defined as amenorrhoea (>6 months) with elevated gonadotropin levels (FSH >40IU/L) before the age of 40 years. The term “premature ovarian failure” was coined by de Moraes-Ruehsen et al. (1967). However, the elevation of urinary gonadotropins in early cessation of menses was reported far before than the above mentioned report (Heller et al. 1939). The clinical symptoms of POF were first described in 1950 among 20 women below the age of 35 years with secondary amenorrhoea, hot flushes, infertility and an atrophic endometrium (Atria et al. 1950). The clinical diagnosis of the disorder along with elevated serum gonadotropins was first reported by Davis et al. (1953). Rebar et al. (1982) described the FSH levels of 26 POF cases cumulatively by using single though arbitrary term “>40IU/L” which is now considered most important clinical measurement for POF diagnosis. Shah et al. (1995) carried out wide range endocrine profiling of POF cases including measurement of serum FSH, LH, Oestradiol, Prolactin, total Thyroxin, total triiodothyronine and TSH. The results revealed extra-ovarian endocrine abnormalities in 49% of POF cases comprising 22% with abnormal thyroid function test, 41% patients tested for adrenocortical reserve showed impaired response of plasma cortisol to adrenocorticotropic hormone stimulation. The patients may sometime regain normal gonadotropin levels, ovulatory functions and even pregnancies have also been reported in approx 7.5% of POF cases (Alper et al. 1986).

Many studies have also reported symptoms of bone mineral loss in the women with POF (Devleta et al. 2004). According to Devleta et al. (2004) the
extent of bone mineral loss is significantly higher in POF cases than normal menopausal and hypogonadotropic women. The early onset of disorder results in higher degree of bone density loss. The rate of occurrence of the POF was reported as 1% in female population by Coulam et al. (1986). Coulam et al. (1986) recruited 1858 women born between 1928 and 1932 from Rochester, Minnesota and found the occurrence of the disorder in 1% of these recruited women.

Kinch et al. (1965) described two histopathological form of POF; the afoolicular and the follicular form. In the afoolicular form, there is complete depletion of follicles and permanent loss of fertility whereas in follicular form few viable ovarian follicles are observed and such women may sometime regain normal function transiently. The follicular form can be subdivided into 1) oophoritis (inflammation of follicles), 2) ovaries with a few follicle present and 3) ovaries with numerous primordial follicles present (resistant ovary syndrome). In POF cases, majority of the ovarian follicles remains in primary or primordial stage with almost absence of mature and ovulatory follicles. The rapid reduction of ovarian follicles occurs due to apoptosis which is specifically termed as 'atresia' for ovarian follicles. The first process of atresia in humans was described by Aron et al. (1953) and the quantitative effect of gonadotropins on atresia was elucidated by Tilly et al. (1991).

In the second half of twentieth century, several reports suggested possible involvement of autoimmune disorders as an important factor in the POF etiology. The role of autoimmune disorders in the etiology of the POF was first suggested by Irvine et al. (1968). Vazquez et al. (1973) further elaborated the association of ovarian failure with autoimmune disorders like
hypoparathyroidism, moniliasis, addison's disease and hasimoto's disease. Coulam et al. (1985) strengthened the role of autoimmune factor by the presence of anti-ovarian antibodies in the serum among 27% of patients from a total of 110 ovarian failure patients recruited for the study. The most commonly associated autoimmune disorder with POF is hypothyroidism. Myasthenia gravis, Crohn's disease, vitiligo and pernicious anemia, like autoimmune disorders are among organ specific autoimmune diseases associated with POF. The systemic lupus erythematosus (SLE) and rheumatoid arthritis are non-organ specific autoimmune disorder associated with POF (LaBarbera et al. 1988). The autoimmune adrenal failure and associated endocrine dysfunction are jointly known as autoimmune polyglandular failure syndrome. In a study, around 3% of POF cases were having polyglandular failure syndrome type I or type II. The type I syndrome manifests as hypoparathyroidism, adrenal failure, chronic mucocutaneous candidiasis and occurs in young children. Majority of type I associated POF cases are primarily diagnosed as primary amenorrhoea. The type II is associated with adrenal failure and hypothyroidism and mainly ovarian failure patients are diagnosed as POF (Betterle et al. 1993). HLA typing of POF cases did not reveal any association of HLA antigens except that this study reported presence of autoimmune antibodies in 43.3% of POF cases and abnormal thyroid function in 23.3% of POF cases (Jaroudi et al. 1994). Fenichel et al. (1997) performed ELISA testing against whole tissue homogenate as antigen, from human ovaries at different ages of 46 POF cases. The circulating ovarian antibodies were detected in 59% of the POF cases (27/46). Within these 27 POF cases, 20/27 were positive for IgG isotype, 9/27 for IgM isotype and 8/27 for IgA. The levels of these ovarian antibodies
were significantly higher in patients than control women. There are even reports of the presence of specific ovarian antibodies in POF patients. Chiauzzi et al. (1982) first reported the presence of an IgG which blocked the binding of FSH with its receptor in two patients with POF and myasthenia gravis. However, subsequent researchers could not demonstrate of such gonadotropin receptor-specific auto-antibodies. Irvine et al. (1969) were the first to report autoantibodies against steroid producing cells. Further studies reported that only a small fraction (< 3%) of POF population was found positive for steroid cell antibodies (Arif et al. 1996, Reimand et al. 2000). Bannatyne et al. (1990) reported the strong association of autoimmune oophoritis with the occurrence of POF. This association was further confirmed by few more researchers.

Nishizuka et al. (1969) reported first mouse model for autoimmune ovarian dysgenesis after the thymectomy of certain mice strain on the day three of life. These mice exhibited oophoritis and progression of anti-ovarian antibodies after puberty. Antiovarian antibodies against zona pellucida, theca cells, granulose cells, ova and steroid cells were detected by week 4 and abated by 14 weeks. Tong et al. (1999) screened ovarian cDNA expression library with the autoimmune serum of postthymectomy mice which developed POF. They found a novel cytoplasmic protein OP1 (ooplasm-specific protein 1) which was specific antigenic target for ovarian autoantibodies. Healy et al. (1985) reported similar aberrant ovarian functions in thymectomized monkey and in human thymic disorders (thymic aplasia and myasthenia gravis).

The first cytogenetic study among ovarian failure patients was reported by Boczkowski et al. (1969). The first study of X-chromosome abnormality in association with ovarian failure was reported by Smith et al. (1974). Krauss et
al. (1987) reported the first familial analysis of women with ovarian failure with interstitial deletion of the long arm of the X-chromosome. They reported 46,XX,del(X)(pter-q21.3::q27-qter) karyotype in all four women within the family, where three women had clear diagnosis of POF while one had menstrual irregularity. Several researchers have been reporting various translocations, deletions in X-chromosome among familial as well as sporadic cases of ovarian failure. The Xq13----q26 was named as critical region and it was hypothesized that any translocation involving this region would lead to ovarian failure or gonadal dysgenesis in early 1970s (Sarto et al. 1973). The significance of association of critical region has been contradictory as of even recently published reports. Many reports confirmed the translocation involving this region (Ferraro et al. 1980, Juberg et al. 1987, Ji et al. 1988, Bates et al. 1990, Tharapel et al. 1993, Prueitt et al. 2000, Marozzi et al. 2000, Rizzolio et al. 2006, Portnoi et al. 2006, Rizzolio et al. 2007). Other group of researchers reported translocations involving critical region without manifestation of ovarian failure (Barnabei et al. 1981, Markovic et al. 1985). Another group reported translocation associated with ovarian failure, involving X chromosome but not the critical region (Rivera et al. 1986). Therman et al. (1990) collected all literature reporting nonmosaic translocation involving X chromosome among infertile women. They concluded that lack of Xp, mainly b region, results in turner's syndrome including gonadal dysgenesis. Their investigation revealed that around 65% and 93% of Xp and Xq translocations/deletions respectively result in ovarian failure. The idic(Xp-) and idic(Xq-) chromosomes always results in ovarian failure. They concluded that the deletions in X-chromosome may cause defects in chromosome pairing and subsequently lead to ovarian
follicular atresia. Davison et al. (1998) reported first study of chromosomal aberrations in a cohort of 79 ovarian failure cases and found one case as XY karyotype and another patient having deletion at Xq26.1. The patient with Xq26.1 translocation had familial history of POF as her mother underwent POF at 28 years with similar deletion. Sato et al. (2004) analyzed the status of skewed X-chromosome inactivation among POF cases which generally gets higher in cases of aberrant X-chromosome (Therman and Patau 1974) including those which cannot be detected by high resolution karyotyping and FISH. X-chromosome inactivation is a phenomenon that occurs in female mammals, such that one of the two X-chromosomes is randomly inactivated to compensate for differences in the X-linked gene dosage between males and females. If inactivation is not random then it results in skewed X-chromosome which can occur due to many factors including X-chromosomal aberrations. The POF cohort had significantly high levels of skewing than control females and five out of 24 POF cases exhibited >90% skewing with its absence in controls.

Gilgenkrantz et al. (1975) reported the first X-autosome translocation associated with the disorder. Ferraro et al. (1980) reported {46,X,Xt(qter leads to p221::p223 leads to qter)} translocation a patient with gonadal dysgenesis and elevated gonadotropins. The first intra-X chromosome insertion was reported by Grass et al. (1981) in a 16 year old female with primary amenorrhoea and elevated gonadotropin levels. Banding suggested that the region q22 through q24 of abnormal X-chromosome was inserted into region p11 of the same chromosome. Several studies also reported the association of autosomal, X-autosomal translocations with POF. Gaal et al. (1977) reported
first unbalanced X-autosomal translocation {45,X,-X,+der(6),t(X;6)} in a patient with primary amenorrhoea, turner phenotype and bilateral streak gonads. Phelan et al. (1977) reported first balanced X-autosomal translocation {46,X,rcp(X;4)(q26;q21)} in a patient with early secondary amenorrhoea and gonadal dysgenesis. Powell et al. (1994) carried out molecular characterization of a balanced X-autosomal translocation [46,X,t(X;6)(q13.3 or q21;p12)] observed in a POF patient. The BrDU analysis showed that normal X chromosome was late-replicating, and translocated X early replicating which is typical of balanced X-autosomal rearrangements. Centre et al. (1994) reported an unbalanced X-autosomal translocation [(46,X,der(X)t(X;6)(q22; p11.2)] in a proband having delayed intellectual and ovarian dysgenesis whereas balanced X-autosomal translocation [46,X,t(X;6)(q22;p11.2)] was present in the proband’s mother with POF. Reciprocal X-autosomal translocation {46,X,rcp(X;11)(q22;q13)} was first reported by Dorus et al. (1979) in a primary amenorrhoea case with elevated gonadotropin levels. The first report of balanced autosomal translocation [(6;15)(p21.3;q15) and a (8;9)(p11.2;q12)] associated with ovarian failure by Tupler et al. (1994).

The elevation of the FSH levels is a landmark clinical feature of POF. The importance of Hypothalamus-pituitary-ovarian (HPO) axis in regulation of FSH secretion and etiology of POF was first reported by Radwanska et al. (1968). The major genes which fall in HPO axis are GNRH (Gonadotropin Releasing Hormone), FSHB (Follicle Stimulating Hormone Beta), LHB (Luteinizing Hormone Beta), FSHR (Follicle Stimulating Hormone Receptor) and LHR (Luteinizing Hormone Receptor). No association study has been carried out for the GNRH and LHR genes with POF. The first mutation study of
the FSHB gene was carried out by Layman et al. (1993) in a small cohort of 18 POF cases and five fertile control women. They sequenced the promoter region and exonic regions of the FSHB gene in one POF case and five control women but could not find any mutation. The mutation screening of the LHB gene was first reported by Suganuma et al. (1995). However, no mutational study could specifically establish the association of the LHB gene with POF. Whitney et al. (1995) reported first genetic analysis of the FSHR though limited to the exon 1, 5, 6 and 10 of the gene. They could not find any associated mutation with ovarian failure except a polymorphism which was located in exon 10 among patients and controls. During the same time of published report by Whitney et al. (1995) and Aittomaki et al. (1995) reported strong association of pAla189Val mutation (c.566C>T) in the extracellular ligand binding domain of the FSHR gene. In vitro expression of mutant receptor in transfected cells exhibited dramatic reduction in binding capacity and signal transduction. However, this mutation was uncommon in other world populations like North American (Layman et al. 1998), Swiss (Jiang et al. 1998), Dutch (Jiang et al. 1998), Singapore Chinese (Jiang et al. 1998), Brazilian (da Fonte et al. 1998), English (Conway et al. 1999), Japanese (Takakura et al. 2001), Mexican (de la Chesnaye et al. 2001) and many more. Two missense mutations p.Asp224Val and p.Leu601Val were reported in a girl having POF. The functional analysis of these mutations resulted in impaired targeting of FSH receptor on membrane (Touraine et al. 1999). Doherty et al. (2002) reported a Finnish female with primary amenorrhoea having compound mutation, c.566C>T and c.1255G>A (p.Ala419Thr). The functional analysis of p.Ala419Thr mutation was found to have minimal effect on ligand-binding capacity and affinity but it almost totally
abolished the cAMP second messenger response. They also screened 40 other Finnish POF women in the same study but neither of the mutations was reported.

Some females in familial Type I galactosemia may also harbor POF disorder. The first report of ovarian failure in galactosemia patients was reported by Kaufman et al. (1981). The etiology of galactosemia is strongly correlated and explained with the deficiency in Galactose-1-phosphate uridyl transferase enzyme. This deficiency may lead to accumulation of galactose and its metabolites which may be toxic to ovarian parenchyma hence women with galactosemia show high incidence of ovarian failure (Kaufman et al. 1981). Histological analysis of ovary in galactosemic women with POF exhibited fibrous ovarian tissue with no intermediate or evolving Graafian follicles (Kaufman et al. 1989). No labeled CO2 production or labeled intermediates were observed among galactosemic women with ovarian failure after incorporation of radio-labeled galactose which otherwise significantly observed in control women. Deficiency in galactose containing compounds or accumulation of galactose-1-phosphate or both may lead to ovarian toxicity and subsequently ovarian failure. Around 229 different mutations have been reported in the GALT gene and many of them have been shown to be associated with galactosemia by a large pool of published studies (Bosch et al. 2006). Most of the carriers for galactosemia as well as patients showed great extent of heterozygosity for the GALT gene mutations. The first genetic analysis of the GALT gene in galactosemic women with ovarian failure was carried out by Fraser et al. (1987) however no genetic association was reported. Kaufman et al. (1993) screened 108 women, heterozygous for the
GALT gene, for their association with ovarian failure. No association could be established between heterozygosity of the GALT gene with the probable incidence of ovarian failure. The screening of the GALT gene was again revisited by a researcher of our lab using most of cases and controls described in the present dissertation and no mutation was found associated with ovarian failure (Kumar et al. 2005).

The significant association of FRAXA premutation and POF is widely established. The FRAXA fragile site is located in the untranslated exon 1 of the FMR1 gene on Xq27.3. The normal FRAXA region is defined by the presence of <60 CCG trinucleotide repeats in the exon 1. FRAXA premutation is defined by the presence of 60-200 repeats and FRAXA full mutation is defined by the presence of >200 repeats. Full FRAXA mutation is associated with the methylation of the FMR1 promoter and subsequent gene silencing which results in mental retardation in males. Earliest study of the association of FRAXA premutation with early menopause was reported by Fryns et al. (1986). The association of FRAXA premutations in defined POF cases unlike early menopause was reported by Conway et al. (1995). Vianna-Morgante et al. (1996) reported a familial case of POF where six females in three generations experienced POF. Conway et al. (1998) again reported FRAXA premutation analysis in 26 women from 23 pedigrees having familial POF as well as 106 sporadic POF cases. Three out of 23 pedigrees (13%) and three out of 106 sporadic cases (3%) had FRAXA premutations. FRAXA premutation was confirmed in three of them where as in one woman carrier status was inferred because of her son having fragile X syndrome. An international collaborative study was performed for fragile X screening among females from familial fragile
X cases. A total of 760 women from such families were surveyed. Among the subjects, 395 carried a premutation, 128 carried full mutation and 237 were non-carriers. Sixty three out of 395 (16%) premutation carriers had experienced premature menopause with none of the full mutation carriers and one from non-carriers (Allingham-Hawkins et al. 1999). Many other studies also supported association of FRAXA premutations with the increased incidence of POF including Marozzi et al. (2000), Gersak et al. (2003), Welt et al. (2004), Machado-Ferreira et al. (2004) and Uzielli et al. (1999). Hundscheid et al. (2000) performed a comparison study between the POF cases with paternally inherited FRAXA premutation (PIP) and POF cases with maternally inherited FRAXA premutation (MIP). They reported that 28% of women with PIP had POF whereas only 3.7% of women with MIP had POF. They suggested the influence of paternal genomic imprinting effect over premutation status. These results were contradicted by Vianna-Morgante et al. (2000) who did not find any association of imprinting effect on risk of POF among premutation carriers. A study was performed on the nearby repeat locus FRAXE for its association with POF and an excess of small alleles with fewer than 11 repeats including at least one small deletion at or near the triplet was found in around 1.5% of POF cases (Murray et al. 1999).

The FOXL2 is well recognized candidate gene in patients with BPES (blepharophimosis, ptosis, and epicanthus inversus syndrome). In BPES type I familial cases, female also inherit POF along with eyelid malformation while in BPES type II the eyelid defect occurs as an isolated entity. Townes PL and Muechler EK (1979) reported the first familial history of co-occurrence of ovarian failure and BPES. First mutation analysis in the BPES gene among 4
females of type I familial BPES syndrome revealed two previously reported autosomal dominant mutations and one new mutation (Smith et al. 1989). The linkage mapping revealed the presence of this gene at chromosomal location 3q22-23 (Amati et al. 1996). Loffler et al. (2003) showed the presence of FOXL2 protein in ovaries starting from the early developmental stages in three distinct vertebrates, mice, chicken and red-eared turtles. The first mutational analysis of FOXL2 gene in type I familial BPES cases where females also inherit ovarian failure was established by Crispony et al. (2001). They studied four such type I familial cases and found the mutations which either resulted in truncation or shortening of the protein. In the first family, a truncation mutation c.892C>T was found which was present across four generations. In the second family, another truncation mutation c.848G>A was found which was present across four generations. In the third family, a duplication of 17bp at position 1092-1108 was found across four generations which caused frameshift mutation resulting in shorter protein. The fourth female was reported as sporadic case. She exhibited c.737-738TC>AA mutation resulting in truncated protein. Harris et al. (2002) carried out mutational analysis of 70 POF cases from New Zealand and Slovenian populations. They found a 30 bp deletion in a Slovenian POF case which resulted in deletion of 10 out of 14 alanines from the polyalanine tract downstream to winged helix/forkhead domain of the FOXL2 protein. One New Zealand POF case was harboring c.772T>A (p.Tyr258Arg) mutation. Rest all remaining patients and 100 controls were not having any mutation. The mutations in this gene were uncommon among Italian cases of POF (Bodega et al. 2004).
The inhibin alpha (INHA) gene is a very important candidate gene for ovarian failure. The serum inhibin levels are significantly lower among POF cases than normal women (Petraglia et al. 1998). Welt et al. (2005) also carried out similar hormonal profiling for FSH, inhibins, and estradiol. They concluded the higher levels of FSH and estradiol with lower levels of inhibins. Shelling et al. (2000) reported the first mutational study of inhibin genes in POF patients. They reported the strong association of c.769G>A (p.Ala257Thr) mutation in INHA gene with its presence in 7% (3/43) POF cases vs. 0.7% in (1/150) control females. Similar association of this mutation were corroborated by Marozzi et al. (2002), Dixit et al. (2004). However, this mutation was found to be rare in Argentine population (Sundblad et al. 2006). The mutational screening was further comprehended for the inhibin receptor, betaglycan (TGFBR3 gene) in gonadotroph cells (Dixit et al. 2006, Chand et al. 2007). The CCAAT haplotype in this gene was found to be associated with ovarian failure.

The BMP15 and GDF9 are oocyte derived growth factors and play major role in growth and differentiation of ovarian follicles. These factors are crucial for cell-cell communication of oocyte with granulosa cell. The first genetic screening of these growth factors was reported by Takebayashi et al. (2000) in Japanese population but no associated mutation was found. Di pasquale et al. (2004) reported a familial POF case harboring p.Tyr235Cys missense mutation in the BMP15 gene. Dixit et al. (2006) reported the presence of eleven different missense mutations in the BMP15 gene with their exclusive presence in Indian ovarian failure patients. Di pasquale et al. (2006) reported the association of some of the similar missense mutations in the BMP15 gene in Italian cohort of POF cases as reported by Dixit et al. (2006). Dixit et al. (2005) reported
c.199A>C (p.Lys67Glu), p.646G>A (p.Val216Mat) missense mutations in the \textit{GDF9} gene associated with ovarian failure. Kovanci et al. (2007) reported p.Pro103Ser mutation in the \textit{GDF9} gene in one out of 61 Caucasian POF cases. Zhao et al. (2007) reported p.Thr238Ala mutation in one out of 100 POF case with its absence in 96 control women. Laissue et al. (2006) reported the presence of p.Leu148Pro mutation in the \textit{BMP15} gene (1/203 POF cases) and p.Ser186Tyr mutation in the \textit{GDF9} gene (1/203 POF cases) in French women with POF. The mutations in both the genes were uncommon in New Zealand population (Chand et al. 2006).

Some other genes like AT2, DIA, NANOS3, KIT, KITLG, \textit{FOXO1A}, \textit{FOXO3A}, \textit{EIF2B2}, \textit{EIF2B4} and \textit{EIF2B5} were also studied for their association with POF. Angiotensin II type 2 receptor is highly expressed in fetus tissue and decreases rapidly after birth. AT(2) receptor is re-expressed in adult atretic ovarian follicles (Katsuya et al. 1997). Angiotensin II type 2 receptor gene (\textit{AT2}) was screened but no mutation was observed. Bione et al. (1998) carried out breakpoint mapping of a familial case of POF harboring a balanced X:12 translocation. They reported that human homology of \textit{Drosophila melanogaster} diaphanous (\textit{DIA}) gene was disrupted in this family due to translocation. This gene is located within critical region (Xq22) and mutated \textit{DIA} gene results in sterility in both male and female Drosophila. Qin et al. (2007) screened the \textit{NANOS3} gene which encodes an RNA-binding protein with conserved role in germ cell development. No mutation was found in these genes among Chinese and Caucasian women with POF. Shibanuma et al. (2002) reported first mutational screening of c-Kit protein gene (\textit{KIT}) in 40 Caucasian POF cases but no mutation was found. Hui et al. (2006) screened KIT Ligand gene
which plays important role in germ cell migration and proliferation. No mutation was found in this gene among 40 studied Caucasian POF cases. Watkins et al. (2006) reported the mutational analysis of two members of forkhead transcription factor family, FOXO1A, FOXO3A, among 90 POF cases from New Zealand and Slovenia. These genes are expressed in ovary like another forkhead member FOXL2. Female Foxo3a knockout mice exhibited POF characteristics with early depletion of ovarian follicles preceded by follicular atresia. Foxo1a protein is a key regulator of the G1/S transition in granulosa cells. The screening of the FOXO3A gene revealed two missense mutations p.Ser421Leu (1/90 POF cases), p.Arg506His (1/90 POF cases) with their complete absence in controls. The screening of the FOXO1A gene revealed p.Pro84Leu in one POF cases with complete absence in controls. Fogli et al. (2003) reported the several missense mutations in the Eukaryotic Initiation factor 2B genes (EIF2B2, EIF2B4, EIF2B5) among ovarian failure patients who were also having central nervous system hypomyelination/vanishing white matter disease leukodystrophy.

Hormone replacement therapy was suggested to be effective in achieving pregnancies in a small fraction of ovarian failure cases. The exogenous administration of vaginal micronized progesterone after estrogen endometrial priming was reported effective for implantation of donor embryo transfer in ovarian failure patients (Fatemi et al. 2007). In the earliest report of administration of Luteinizing hormone releasing hormone inhibitor (goserelin) which resulted in significant reduction of gonadotropin levels but remained ineffective in normalization of POF patients (Ladger et al.1989). Another study demonstrated that the administration of buserelin (luteinizing hormone
releasing hormone inhibitor) and Human Menopausal Gonadotropin (HMG) independently improved the stimulation of ovaries, embryological and clinical outcome for in vitro fertilization, with a success rate of more than 75%, among ovarian failure cases (MacLachlan et al. 1989). The ovarian stimulation effects of buserelin, HMG by another independent study could not establish similar observation in group of ovarian failure cases (Surrey et al. 1989).

Several mouse models have been generated to elucidate the etiology of POF. Some of these models exhibit similar histology and ovarian aberrations quite similar to human POF pathophysiology whereas many other models exhibit ovarian dysgenesis/female infertility unlike POF. We have not described those mice genetic models where ovarian dysgenesis/infertility is merely an associated feature rather than primary abnormality. The generation of c-kit deficient mice was the first report which exhibited complete germ cell loss due to the defect in migration and proliferation of germ cells (Coulombre et al. 1954). Similar defects were observed in kit ligand deficient mice (Bennett et al. 1956). The mechanism/physiology of germ cell deficiency was different in c-kit and kit ligand knockout mice than POF. The first knockout mouse as a model for POF was developed for Gcd (germ cell deficient) gene by insertional mutation (Pellas et al. 1991). The physiological effects of the mutation were limited to reproductive system. Both male and female homozygous mutants were infertile. The germ cells were almost absent in homozygous mutants and significantly reduced in heterozygous mutants. The germ cells and oocytes were seen at birth but most of the oocytes undergo rapid atresia after birth resulting in very few oocytes which reach maturation and ovulation. Though gonad size remained normal in both heterozygous as well as homozygous
mutants as compared with normal mice but ovarian senescence was observed histologically in mutant mice. The GDF9 is a member of transforming growth factor β family which are exclusively synthesized and expressed by oocytes. The expression of GDF9 is initiated by oocytes from one layer follicle stage and is continued until after ovulation. Dong et al. (1996) generated the Gdf9 knockout mice causing infertility. The size of ovaries was much smaller in knockout female mice than wild type. These female knockout mice fail to demonstrate any normal follicle beyond the primary one layer follicle stage. These mice show complete lack of corpora lutea. Follicles beyond one layer follicle stage in the ovaries are abnormal and contain asymmetrical arrangement of atypical granulosa cells. These knockout mice had 2-3 times higher gonadotropin levels than wild type similar to POF. The zing finger protein X gene (ZFX) is located in critical region of X chromosome in human. This protein plays role in oogenesis, follicle development and maintenance of fertility. The female knockout mice for the Zfx gene were small in size, less viable and had fewer germ cells. The reduced number of oocytes resulted in diminished fertility and shortened reproductive lifespan similar to POF in human (Luoh et al. 1997). The ovarian histology of mutant female mice was reminiscent as observed in POF. However, the Zfx gene does not seem to affect the fate of reproductive tract and genitalia. Connexin37 is a gap junction protein which helps in cell-cell communication between oocytes and granulosa cells. The female knockout mice for Connexin37 (Cx37) gene shows lack of ovulation and Graafian follicles (Simon et al. 1997). Histological analysis showed complete absence of oocytes-granulosa cell gap junctions and connexin37. Occasionally antral follicles were seen in these female mice but
they never developed into Graafian follicles. These mice also showed 5-10 times higher count of corpora lutea than normal mice which were even much smaller in size. The Foxl2 knockout mice also exhibited few features similar to POF (Uda et al. 2004). These female mice were smaller in size with craniofacial features comparable to BPES. Ovary size was reduced and tubes were hypotrophic with incomplete glandular cytodifferentiation. Histological analysis of ovaries revealed the major presence of follicles surrounded by monolayer or occasionally two layers of granulosa cells. Epithelial cells were pleiomorphic, including a few flattened cells reminiscent of primordial cells. Follicular growth was severely impaired till early its stages as described above. The atretic follicles were completely absent in mutant mice at any age. The female knockout mice for the Nobox gene also showed features comparable to POF (Rajkovic et al. 2004). Nobox is oocyte-specific homeobox gene expressed in germ cell cysts and in primordial and growing oocyte. The homozygous mutant female mice were infertile with atrophic ovaries that lacked oocytes at 6 weeks. The ovarian development was similar in mutant mice and wild type mice in terms of germ cell proliferation, primordial follicle formation and histo-morphology. Postnatal ovaries have mainly ovarian follicles in primordial stage with almost absence of antral and later stages. There is steep loss of oocytes after birth. By the day 14, very few oocytes were visualized and most of them were degenerating. Overall the Nobox homozygous mutant mice were infertile along due to blockage of follicle development and degeneration of follicles. Oocytes are continuously recruited to re-acquire meiotic competence at follicular development stage. Meiotic arrest is maintained in competent oocytes until the luteinizing hormone surge which causes ovulation and leads
to progression of meiosis to Metaphase II. The cAMPs play major role in such meiotic arrest through the involvement of G proteins and G protein coupled receptor GPR3 specifically located on oocytes. The GPR3 receptor prevents premature resumption of meiosis in antral follicles. The female Gpr3 knockout female mice exhibited accelerated Age-dependent reduction of fertility (Ledent et al. 2005). The knockout females gave birth of smaller litters with increasing age of maternity. During culture of Gpr3−/− oocytes only 50% of oocytes resume meiosis as compared with 80% in wild type. Overall, these above presented mice models have elaborated the physiological outcomes in their absence. Many of these genes have been studied in human cases for their germline status/protein activity/quantitative analysis.