Chapter- 2

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CHAPTER 2

REVIEW OF LITERATURE

The reproductive traits play a large role in determining the efficiency of production in livestock species which makes reproduction one of the most extensively studied areas of animal agriculture. Genetic variation of quantitative traits such as reproductive efficiency; growth rate, milk yield etc. are controlled by many genes, each with a small effect. These genes influencing quantitative traits can have different effects (Geldermann et al., 1985). However, the major gene model suggests that a few genes may account for relatively larger proportion of genetic variation. Genes involved in the biology of a trait are candidates for association study. One such class of genes encodes for hormone-receptor super family. Hormone receptors are ligand-activated transcription factors that act as the link between the hormone and a physiological response.

The present investigation was carried out on “Molecular characterization of major candidate genes associated with reproductive traits in buffalo (Bubalus bubalis)” and the literature related to this subject has been reviewed under the following heads:

Molecular characterization of estrogen receptor gene.

Molecular characterization of progesterone receptor gene.

2.1 Molecular characterization of estrogen receptor gene

Estrogen is an intra ovarian factor affecting reproduction and the function of hypothalamus, pituitary, liver, skeleton and calcium homeostasis (Huseyin Baki, 2013). It also influences growth, differentiation and function of reproductive tissues like mammary gland, uterus, ovary, testes and prostate. Furthermore, estrogen was found to increase sexual behaviour, regulate secondary sexual traits and play a major role in female post-natal physiology and pathology (Zahmatkesh et al., 2011). Estrogen is named for its importance in the estrous cycle. Animal body naturally produce three main forms of estrogen, which are estradiol17P (E$_2$), Estrone (E$_1$) and Estriol (E$_3$). In the ovary, E$_2$ is the most physiologically active type of estrogen produced by granulosa cells of pre-ovulatory follicles through the aromatization of thecal androgen by the granulose cells of growing follicles. In the testis, some E$_2$ is produced by Sertoli cells through the aromatization of the
androgen synthesized from the Leydig cells. Estrogen is also synthesized in extragonadal sites including the mesenchymal cells of adipose tissue and skin, osteoblasts and chondrocytes of bone, vascular endothelium and aortic smooth muscle cells as well as several sites in the brain, including the anterior hypothalamus and the medial basal hypothalamus (Bayard et al., 1995; Simoni et al., 2002).

Estrogen exerts their effect on target cells through protein receptor called estrogen receptor (ESR). Estrogen receptors like other members of nuclear receptors' super family, are transcription factors which bind to estrogen and regulate gene transcription (Szreder and Zwierzchowski, 2004a; Szreder and Zwierzchowski, 2004b; Jakimiuk et al., 2007). There are two isoforms known for estrogen receptor: ESR-a and ESR-P each encoded by separate genes located in different chromosomes. The major sites of ESR expression are reproductive organs and also stomach, kidney, lung, liver, intestine and pituitary (Zahmatkesh et al., 2011).

Estrogen signalling is selectively stimulated or inhibited depending upon a balance between ESR a and ESR P activities in target organs. ESRs have five distinct regions corresponding to functional and structural units called domain. The N-terminal of the A and B domains are highly conserved only between chicken and human estrogen receptors, but this distinction is much less clear in the other steroid Receptors. Therefore, region A and B are combined into A/B region in most cases. This region is a modulatory region and it is the most variable both in size and sequence. The A/B domain consists of Activation Function 1 (AF1), which contributes to the transcriptional activity of ERs and is an essential domain for interaction with co-regulators. The C domain is DNA Binding Domain (DBD) and the most conserved region of the estrogen receptors. It is essential for sequence specific binging of ESRs to DNA. The D domain is not well conserved among the different receptors and serves a hinge between the DBD and the Ligand Binding Domain (LBD) allowing rotation of the DBD. Domain E is the Ligand Binding Domain (LBD), which contains COOH-terminal AF-2 motif responsible for ligand-dependent transcriptional activation. The F region is essential for hormone binding in the ESR a (Skafar and Zhao, 2008).

Estrogen receptor a was found expressed in the uterus, vagina, hypothalamus, and theca cells of the ovary. In contrast, ESR P was primarily expressed in the prostate, cerebral cortex, and granulosa cells of the ovary (Kuiper et al., 1997; Osterlund et al.,
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2000). The localization of ESR P to the cerebral cortex led researchers to speculate that the receptor might also play a role in the regulation of cognition, motor skills, and memory. In order to study in more detail the physiological effect of ESR P, transgenic mice lacking ESR P were developed (Krege et al., 1998). Estrogen receptor P knockout mice showed a phenotype distinct from that of ERα knockout mice. While ESR α knockout animals showed gross defects in both reproductive tract morphology and mating behaviour (Lubahn et al., 1993), ESR P knockout animals developed normally and showed no defects in mating behaviour. However, ESR P knockout females did produce fewer and smaller litters than wild-type females, primarily due to reduced ovarian efficiency. This reduced ovarian efficiency might be due to the loss of ESR P function in granulosa cells of the ovary. Prior to the development of ESR α knockout mice, it was thought that estrogen receptor was necessary for survival. The subsequent discovery of the ESR P gene led researchers to believe that the presence of either form of the estrogen receptor was sufficient for survival. While from these studies it was recognized that both ESR α and ESR P were required for ovarian function, the exact mechanism of these actions were not yet well understood (Krege et al., 1998).

Andersen et al., (1994) compared the allele frequencies of three restriction fragment length polymorphisms at the oestrogen receptor (ESR) locus between breast cancer patients and controls. Alleles having the XbaI restriction site detected by the M72 probe (covering exon 2 and flanking introns) were found significantly more frequent in patients than in controls (P = 0.033). Within the breast cancer population, the presence of the XbaI restriction site was associated with late onset of the disease but this association was only of borderline significance. The allele frequencies of the BstUII polymorphism in exon 1 and the PvuII polymorphism in intron 1 did not differed between cases and controls. However, alleles with the PvuII restriction site were more frequent in patients with progesterone receptor negative primary tumours than in patients with progesterone receptor positive primary tumours (P = 0.027). Furthermore, no significant association was observed between any of the ESR polymorphisms and the oestrogen receptor status of the primary tumours.

Korach et al., (1996) disrupted the ER gene and produced a line of transgenic mice possessing the altered ER gene (ERKO) using the techniques of homologous recombination. The mouse ER gene was disrupted by inserting a 1.8 kb PGK-Neomycin
sequence into exon 2, approximately 280 bp downstream of the transcription start codon. Estrogen insensitivity was confirmed using estrogen agonists, estradiol, hydroxy tamoxifen, diethylstilbestrol treatment for 3 days which resulted in a 3-4-fold increase in uterine wet weight and vaginal cornification in wild-type females, while ERKO mice remained totally unresponsive. The data was further supported by the failure of estrogen or EGF treatment to induce DNA synthesis in uterine tissue of similarly treated mice. Wild-type mice treated with a single estradiol injection showed a 350-fold induction in lactoferrin mRNA. Both male and female animals survived to adulthood with normal gross external phenotypes. Although the reproductive capabilities were altered with a dramatic effect on the gonads, prenatal development of the reproductive tracts of both sexes appeared to be independent of an ER-mediated response.

Georgiou et al., (1997) analysed a common *Pvu*II and a rare *Bst*UI polymorphism in the ESR gene of women undergoing ovarian stimulation for in-vitro fertilization (IVF) and embryo transfer and controls having at least one pregnancy. Pregnancies from IVF were observed significantly rarer in patients who were homozygous for the *Pvu*II polymorphism (*P* > 0.05). Frequencies of *Pvu*II positive (P) and negative (p) ESR alleles, present in the IVF group, did not differ significantly with the control group. The frequency of *Bst*UI positive (B) alleles was low in the IVF and control groups and so was in the Bb genotype. Only one BB genotype was found in the IVF group while none was found in the control group. The observed haplotypes were p-b, P-b, p-B and P-B, with equal frequencies in both groups with the exception of haplotype P-B (having both *Pvu*II and *Bst*UI restriction sites) which was found only in the IVF group. Most pregnancies in the IVF group were found in the pp (46%) and the Pp (46%) genotypes and only three in a total of 37 pregnancies (8%) were found in the PP genotype. This distribution of genotypes was statistically different from that in both the IVF patients and the control group (*P* > 0.05). The ESR B variant, which was analysed using the restriction enzyme *Bst*UI, was not found to have an influence on the numbers of the follicles, oocytes and ratios, when compared alone or in combination with *Pvu*II.

Hori et al., (1998) assessed the ER variants in human various breast tissues and investigated the correlation between the expression of ER variants and ER/progesterone receptor (PGR) status. The frequencies of 3 ER splice variants (del 2/3, del 5, and del 7) were assessed in 24 benign and 76 malignant breast lesions by the reverse transcription-
polymerase chain reaction (RT-PCR) method and compared with histopathological features. Deletions of exons 5 and 7 were more frequent than the deletion of exon 2/3 in both benign and malignant lesions, and the expression of del 5 and del 7 seemed to correlate with epithelial overgrowth. There was no significant difference between the ER/PGR protein status and the expression of each ER variant in breast cancer. ER and PGR proteins were analyzed using enzyme immunoassay (EIA) and immune histochemical assay (IHA) in 68 cases of cancer to determine whether aberrant variants influence the status of either protein. The expression of ER splice variants was not uncommon in human breast diseases. The abnormal ER structures were not considered to be a main factor for tamoxifen insensitivity or important regulators of PGR expression. Further, no correlation was observed between any of the splice variants and the status of either protein. The results of the mRNA analysis of the four protein status groups showed the same trend, indicating that the frequency of hormone binding domain deletion was higher than that of DNA-binding domain deletion.

Rosenfield et al., (1999) amplified, cloned and sequenced the bovine ERP (bER P) by reverse transcription-polymerase chain reaction (RT-PCR). The open reading frame of bER P cDNA was found to span 1584 nucleotides encoding a protein of 527 amino acids. The N-terminal region of bER P was found to be 80% homologous to human and mouse ER P and 79% homologous to rat ER P. Bovine ER P DNA binding domain was 100% homologous to human, mouse, and rat ER P sequences. The C-terminal/ligand-binding domain of bER P was 89% homologous to human, 86% homologous to mouse, and 88% homologous to rat ER P. Human and bovine ER P amino acid sequences were similar in that their coding region extended farther 59 than initially reported for the published rat ER P sequence.

Kaminski et al., (2002) presented a method allowing simultaneous genotyping of two loci: ryanodine receptor 1 (RYR1) and estrogen receptor (ESR). A total of 122 Polish Large White and Polish Landrace pigs were genotyped by this method, demonstrating its reliability, convenience and lower costs. This method might be useful in the wide-scale genotyping of both loci in pig breeding programmes. In multiplex PCR amplification, two amplicons were simultaneously produced: a 272 bp fragment of RYR1 gene and a 185 bp fragment of ESR. After the sequence analysis it was found that both PCR products could
be subjected to simultaneous digestion with *Hin6* I and *Ava* I, which resulted in a combination of bands enabling the identification of RYR1 and ESR genotypes.

Herrington *et al.*, (2002) characterized 309 women with coronary artery disease who were enrolled in the estrogen replacement and atherosclerosis trial with respect to eight previously described and two newly identified ERα polymorphisms and examined the association between these polymorphisms and the response of HDL cholesterol and other lipids to treatment with estrogen alone or estrogen plus progestin. After adjustment for age, race, diabetes status, body-mass index, smoking status, alcohol intake, and frequency of exercise, the 18.9 percent of the women who had the IVS1-401 C/C genotype had an elevation in the HDL cholesterol level with hormone-replacement therapy which was more than two folds the increase observed in the other women; this effect was found limited to changes in the HDL sub-fraction 3 (HDL3). Similar patterns of response were also observed for three other highly linked ERα intron 1 polymorphisms close to the IVS1-401 site. The pattern of increased response of HDL cholesterol in women with the IVS1-401 C/C genotype was evident in both the women receiving estrogen and those receiving estrogen plus progestin and was significant among women who were compliant with the study medication (*P*<0.001). Hence the postmenopausal women with coronary disease, having the ERα IVS1–401 C/C genotype, or other closely related genotypes, had an augmented response of HDL cholesterol to hormone-replacement therapy.

Kaminski *et al.*, (2003) evaluated the possible relationship between ESR/Ava I polymorphism and carcass performance traits in Polish Large White boars like daily gain, meatiness and selection index. 103 boars originating from one herd in NE Poland were examined and identified three ESR/Ava I genotypes: 33 WW, 35MM and 35WM. ESR/Ava I genotypes were determined by the PCR-RFLP method. All boars were genotyped by our multiplex PCR method for simultaneous genotyping of RYR1 and ESR loci. A highly significant differences (*P* < 0.01) between WW and MW genotypes as well as significant differences (*P* < 0.05) between WW and MM genotypes was found by the use of the Duncan test. No significant differences were observed for daily gain and selection index. The highest values of meatiness were observed in WM boars (61.21%). Since boars were kept in the same environmental conditions and showed a relatively high level of production traits, the relation described in this work indicated the positive
influence of ESR/Ava I polymorphism on meatiness and therefore supported its potential usefulness in pig breeding programs.

Tanaka et al., (2003) examined the distribution and morphology of immuno reactive estrogen receptor alpha (ERα) containing cells in the cattle brain. The brains were collected from two cows and a male calf. One of the cows was sacrificed one week after parturition. ERα expressions in the preoptic area of the rostral forebrain and the medial basal hypothalamic area were evaluated immuno histochemically using a mouse monoclonal antibody. In all animals, the signals that indicate ERα were detected in the medial preoptic area, the level of the organum vasculosum of the lamina terminalis and the bed nucleus of the stria terminalis. In the caudal hypothalamic region, they were detected in the arcuate nucleus, the periventricular and ventromedial hypothalamic nuclei. ERα-containing cells were characterized by a dense nuclear reaction product. ERα signals were clearly detected in the cytoplasm as well in all the subjects examined. The density of ERα expression was different among the three cattle; the number of ERα containing cells in the postpartum cow was much lower than that in the other cow and the male calf. ERα expression in the forebrain was suggested to be influenced by the reproductive status in the cattle.

Meurs et al., (2003) analysed three polymorphic sites in the 5' region of the ESR1 gene; a (TA)$_n$ repeat in the promoter region, molecular haplotypes of the PvuII and XbaI RFLPs in intron 1 and inferred long-range haplotypes (LRH). Only three of the possible four PvuII–XbaI haplotypes were observed in population considered. The (TA)$_n$ VNTR was located at a distance of 35 kb from the two RFLPs so molecular haplotypes of all three polymorphisms could not be easily determined. The frequencies of the haplotypes were found similar in other Caucasian populations, but in two Asian populations haplotype 3 (Px) was more frequent, while the haplotype 2 (PX) allele was less frequently observed. In the small African population tested (Coriell panel), haplotype 1 was less frequent than in Caucasian and Asian women, and haplotype 2 was more frequently present. In all populations the haplotype 4 (pX) was rare or not present at all. In men, no association was observed between the ESR1 polymorphisms studied and bone parameters whereas in women, an allele dose effect of haplotype ‘px’ (P=0.003) and a low number of (TA)$_n$ repeats (P=0.008) with decreased lumbar spine bone mineral density (BMD) along with decreased vertebral bone area were demonstrated. Furthermore, an increased
vertebral fracture risk with evidence for an allele dose effect with an odds ratio of 2.2 (95%; CI 1.3–3.5) for haplotype ‘px’, and 2.0 (1.5–3.2) for a low number of (TA)\textsubscript{n} repeats was also found. The ESR1 genotype dependent fracture risk was found to be largely independent of BMD and bone area. ESR1 polymorphism in the 50’ (promoter) region was found to be associated with vertebral fracture risk, lumbar spine BMD and vertebral bone area in postmenopausal women, but not in men. Furthermore, linkage disequilibrium (LD) analysis between the \textit{PvuII–XbaI} haplotype and the (TA)\textsubscript{n} repeat showed strong LD between the two sites. However, the largest effect was always observed for the LRH allele A, which was the combination of a low number of TA repeats and \textit{PvuII–XbaI} haplotype 1.

\textit{Lu et al.}, (2003) showed that the estrogen receptor alpha (ER a) mediated the effects of estrogen by altering gene expression followed by the hormone binding. It was shown that kinase-mediated phosphorylation of ER a also transcriptionally activated the receptor in the absence of estrogen. The ER a -dependent gene expression was also reported to be regulated by protein phosphatase 2A (PP2A). ER a co-immunoprecipitates with enzymatically active PP2A. ER a was reported to bound directly to the catalytic subunit of PP2A, which further dephosphorylated serine 118 of the receptor. Amino acids 176-182 in the A/B domain of ER a were required for the interaction between PP2A and the receptor. Phosphatase inhibition disrupted the ER a -PP2A complex and induced the formation of an ER a -activated mitogen-activated protein kinase complex, phosphorylation of ER a on serine 118 and transcriptional activation. These findings demonstrated that estrogen receptors existed in complexes with phosphatases as well as kinases.

\textit{Suzuki et al.}, (2003) evaluated the association between the risk of developing familial prostate carcinoma and genetic polymorphisms in estrogen-related enzymes and receptors. One hundred one cases with prostate carcinoma whose first degree relatives had prostate carcinoma and 114 healthy age and residence matched male controls were studied and analysed the genotypes of estrogen receptor (ER) alpha, aromatase (CYP19), and catechol-O-methyltransferase (COMT) genes. The number of high-risk genotypes (the T/T in ER alpha, the C/T and T/T in CYP19, and the G/A in COMT) significantly increased the risk of prostate carcinoma development (2 genotypes: OR, 3.00; 95% CI, 1.72–5.23; \textit{P}= 0.008; 3 genotypes: OR, 6.30; 95% CI, 3.61–10.99; \textit{P}= 0.002). Genetic polymorphisms
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of genes in the estrogen metabolism pathway were found in association significantly with familial prostate carcinoma risk. Single nucleotide polymorphisms of low-penetrance genes were considered to be the targets for understanding the genetic susceptibility of familial prostate carcinoma. Four genotypes of estrogen-related substance genes were evaluated among control patients and the ER alpha \textit{Pvu}II genotypes of C/C, C/T, and T/T were observed in 22.8%, 51.8%, and 25.4%, respectively, whereas the C/C, C/T, and T/T genotypes were observed in 11.8%, 42.6%, and 45.5% of case patients, respectively. The T/T genotype of ER alpha \textit{Pvu} II was associated significantly with the risk of developing prostate carcinoma (OR, 3.44; 95% CI, 1.97–5.99; \(P=0.0028\)). In contrast, no significant differences were found in the distribution of the ER alpha \textit{Xba} I genotypes.

Okura \textit{et al.}, (2003) examined the association of polymorphisms of the estrogen receptor (ER) a gene with body fat distribution in a total of 2238 community-dwelling middle-aged and elderly Japanese population (age: 40–79 years). A significant and inverse correlation \((r=-0.630, P<0.001)\) was found between FM (fat mass) and age in the group with the GG genotype. Waist circumference was positively associated with age \((r=-0.231, P<0.001)\) in the group with the AA genotype, whereas an inverse association \((r=-0.504, P<0.001)\) was found in the group with the GG genotype. In particular, those with the GG genotype had a 9% greater BMI, a 20% greater FM and a 9% larger waist compared with the AA genotype. However, no association was found between the ERa gene polymorphisms and body fat distribution in men. No difference was found in BMI (body to mass index), waist, hip or WHR (waist to hip ratio) between genders, whereas %FM and FM were significantly greater in women than in men. The distributions of ERa genotypes with regard to the T-C and A-G SNPs were found in Hardy–Weinberg equilibrium. The distribution of haplotypes for the T-C and A-G polymorphisms in all subjects was as follows: T/A, 61.9%; T/G, 0.2%; C/A, 28.6%; and C/G, 9.3%. The T-C and A-G SNPs were reported to be in linkage disequilibrium. For both genders, no significant difference was found in any variables among the T-C polymorphisms (TT, TC and CC genotypes) with the exception of BMI of the older men (60–79 years).

Lee \textit{et al.}, (2004) evaluated the genetic influence of \textit{Pvu}II and \textit{Xba}I polymorphisms of oestrogen receptor a (ERa) in patients with systemic lupus erythematosus (SLE) in Korea. \textit{Pvu}II and \textit{Xba}I restriction fragment length polymorphism was used to analyse genomic DNA from 268 female controls and 137 female SLE patients.
(41 childhood onset and 96 adult onset) were analysed using and the frequencies of alleles and genotypes were compared in control and patient groups. The Pp genotype was observed more often in SLE patients than in controls (p(c) = 0.017), ERα genotype distributions of adult onset SLE did not differ significantly from controls. The PP, Pp, and XX genotypes were found less often in childhood onset SLE (p(c) = 0.0045, 0.0498, and 0.0255, respectively) than that in controls. Additionally, the PP genotype was less common in childhood onset than in adult onset SLE (p(c) = 0.016). SLE patients with the PP genotypes were older at disease onset than those with the other genotypes (p = 0.001). Patients with the Xx genotype had an earlier onset of SLE than those with the xx genotype (p = 0.025). The frequency of the combined ppXx genotype was found higher in childhood onset SLE than in controls (p(c) = 0.0009) or adult onset SLE (p(c) = 0.027). The same trend was further supported by the subgroup analyses according to age at menarche and logistic multivariate analyses. ERα polymorphisms were significantly found in association with the age at disease onset in Koreans with SLE.

Schuit et al., (2004) studied the association of ESR1 haplotypes created by the c.454-397T>C (PvuII) and c.454-351A>G (XbaI) polymorphisms with myocardial infarction (MI) and ischemic heart disease (IHD) risk in 2617 men and 3791 postmenopausal women (aged 55 years and older). Detailed interviews and physical examinations were performed, blood samples were obtained and cardiovascular risk factors were assessed. Approximately 29% of women and 28.2% of men were homozygous carriers of the ESR1 haplotype 1 (-397 T and -351 A) allele, 49% of women and 50% of men were heterozygous carriers, and 22% of women and 21.4% of men were non-carriers. During a mean follow-up of 7.0 years, 285 participants (115 women; 170 men) had MI, and 440 (168 women; 272 men) had an IHD event, of which 97 were fatal. After adjustment for known cardiovascular risk factors, female heterozygous carriers of haplotype 1 had an increased risk of MI (event rate, 2.8%; relative risk [RR], 2.23; 95% confidence interval [CI], 1.13-4.43) compared with non-carriers (event rate, 1.3%), whereas homozygous carriers had an increased risk (event rate, 3.2%; RR, 2.48; 95% CI, 1.22-5.03). For IHD events also a similar association was observed. In men, the ESR1 haplotypes were not associated with an increased risk of MI (event rate, 5.7%; RR, 0.93; 95% CI, 0.59-1.46 for heterozygous carriers; and event rate, 5.1%; RR, 0.82; 95% CI, 0.49-1.38 for homozygous carriers) compared with non-carriers (event rate, 5.8%) and were not associated with an increased risk of IHD. The postmenopausal women who carry
ESR1 haplotype 1 (c.454-397 T allele and c.454-351 A allele) was found to have an increased risk of MI and IHD, independent of known cardiovascular risk factors whereas no association was observed in men.

Kossakowska et al., (2004) assessed the insulin-like growth factor 1 (IGF1) and estrogen receptor (ESR) genes as candidate genes in 53 F1 sows (Zlotnicka Spotted boars × Polish Large White sows) for total number of piglets born (TNB), number of piglets born alive (NBA), litter weight at birth (LWB), litter weight at weaning (LWW), number of piglets weaned (NPW), average piglet weight at weaning (AWW) and gestation length (GL). The IGF1 locus genotype (microsatellite sequence) was found to affect (P < 0.01) the TNB, NBA and GL when based on analysis of sows with all litters born. Further three point mutations were also detected at ESR locus. Two in exon 8 where a T→C mutation at position 1665 creates an AvaI restriction site, and A→G mutation at position 1756 creating MspI restriction site. The third mutation, detected by PvuII restriction enzyme was located in an intron with two alleles – “A” (120 bp) and “B” (65 and 55 bp) with the frequencies of 0.7 and 0.3. No association appeared between reproduction traits and ESR genotype in any of the observed restriction sites. For the ESR/MspI polymorphism for TNB, NBA, LWB, LWW and NPW, animals of the genotype 5/6 showed non significant trends for a lower reproductive efficiency than those of 5/5 or 6/6 genotype.

Goliasova and Wolf (2004) evaluated the effect of PvuII polymorphism of the oestrogen receptor gene on litter size and production traits in a Czech Large White population (882 sows, 2,455 litters) by four-trait animal models. The traits analysed were lifetime average daily gain in the field test, lean meat percentage, number of piglets born alive in parity 1, number of piglets born alive in parity 2 and subsequent parities. Two variants were calculated for each model i.e. the ESR effects either across herds or within herds. On the genetic level, no significant overall effects of the ESR gene were detected and the effects of the ESR gene within herds were observed mostly insignificant. However, when considering the phenotypic means of the production traits for the ESR genotypes in individual herds, a tendency of better performance connected with allele B was evident for lean meat percentage. There was a certain tendency for allele A to increase litter size in parity 1. In some herds, relatively marked differences between the results for Model 3 and Model 4 were observed. These differences might be caused by low numbers
of observations in the given herds like in herd 1 and 6. Another reason might be an unfavourable distribution of the ESR genotypes within the given herd.

Szreder and Zwierzchowski (2004a) amplified 5′ region of ER a gene by designing primers on the basis of the sequences of the human, ovine, and porcine ER genes. Seven overlapping fragments of the 5′ region of the bovine ERα gene were amplified and sequenced. Altogether, these fragments were composed in the 2853-bp sequence which included the noncoding exons A, B, C, their putative promoters and a part of the coding exon 1. A polymorphism was identified for the first time within the 5′ region of the bovine ER a gene i.e. the A/G transition, which could be recognized with RFLP-Bgl I, lying upstream to the exon C. The Bgl I cut the 242-bp amplification product into 182-bp and 60-bp fragments for allele G, while allele A remains uncut, resulting in two restriction fragments for homozygote GG and three fragments for the heterozygote AG. The polymorphic site was located 254 nucleotide upstream exon C and 2429 nucleotide upstream the putative transcription start site of exon 1. The sequences of the 5′-regions of the bovine, murine, porcine, and human ER a genes were compared. The alignment of the bovine and porcine genes showed 87% similarity. Both genes differed by the 23-nucleotide deletion/insertion (pos. 115 – 138 in the bovine gene), and a second long (29-nt) deletion in the bovine ER a gene (pos. 1699-1728). The former deletion was also found in the human ER a gene, while the latter seems typical for the bovine gene, although in the porcine ER a gene a shorter deletion (18-nt) was found at this position. The polymorphic gene variants were first identified with the PCR-HD method, which relies on differences in the hybridisation of complementary and non-complementary DNA strands, leading to the formation of different heteroduplex (HD) forms (Wang et al. 1992). The HD variants 1 and 2 correspond to the GG homozygote and AG heterozygote, respectively.

Szreder and Zwierzchowski (2004b) reported a single-strand conformation polymorphism (SNP) by PCR-RFLP with TspRI restriction endonuclease within the coding region of the bovine estrogen receptor ER a gene. Within the analysed population of cattle, three different highly reproducible SSCP patterns were observed. The DNA samples were sequenced representing SSCP patterns 1 and 2 (putatively homozygotes). A nucleotide substitution, the A→C transversion was identified at nucleotide 503, upstream the putative transcription start site of the exon 1. The SNP was located within the proline CCA codon. However, this mutation does not change the amino acid sequence of ER a
protein, since both triplets – CCA and CCC – code for proline. Comparison of the restriction maps of both ER α gene variants revealed that A>C substitution created a restriction site for TspRI endonuclease, thus enabling PCR-RFLP analysis of the gene polymorphism to be performed. The following restriction fragments resulting from digestion of the 294 bp PCR product with TspRI endonuclease were found: the uncut 294-bp amplicon for genotype CC, 277-bp and 17-bp bands for genotype AA, and 294-bp, 277-bp, and 17-bp for heterozygotes AC. This was the first ever report on the polymorphism in the protein-coding sequence of the bovine ER receptor gene.

Omelka et al., (2005) investigated the effect of oestrogen receptor (ESR) gene on total number of born (TNB), number of born alive (NBA) and number of weaned (NW) piglets in Large White (LW), White Meaty (WM) and Landrace (L) sows from six Slovak breeding farms. Detection of ESR (Pvu II) genotypes was performed by the PCR-RFLP method. The frequencies of favourable B allele in LW, WM and L were 0.33, 0.25 and 0.08, respectively. In WM, a positive association of B allele with TNB, NBA and NW was found but the differences were not confirmed statistically. There was a highly significant effect of ESR locus on NW (P < 0.01) in LW, however, in contrast with other studies a negative effect of BB genotype on the trait was also observed. This could be probably due to higher dependence of NW on environmental factors. The AB genotype showed a tendency to improve other tested traits but the results were not statistically significant. A highly significant effect of ESR locus on TNB, NBA and NW (P < 0.01) was identified in L breed. An increase by +0.62 ± 0.18 (TNB), +0.65 ± 0.18 (NBA) and +0.51 ± 0.16 (NW) pigs per copy of B allele was found. The BB genotype was not evaluated because of its absence. It was also possible that the differences could be a consequence of dominant effect. The results further showed the possibility of ESR utilization in marker-assisted selection to increase litter size only in Landrace pigs.

Schuit et al., (2005) determined ESR1 PvuII-XbaI haplotypes in 631 postmenopausal women and 528 men (aged 55 years and above) and correlated them with plasma E2 levels. Extreme genotypes, representing 23 % and 27 % of the population, varied by 3.93 pmol/l and no association with plasma testosterone was observed. In a subset of 446 women, no association of genotype with plasma concentrations of dehydroepiandrosterone sulfate, androstenedione or estrone was seen and in men also none of the sex hormone levels was associated with the ESR1 PvuII-XbaI haplotypes. A
role for genetic variations in the ESR1 gene was demonstrated in determining postmenopausal E2 levels in women. A significant association was observed between E2 levels and age in both men and women. The four possible $Pvu$II-$Xba$I haplotype alleles were observed in the following frequencies: haplotype 1 (T-A) 52.5%, haplotype 2 (C-G) 35.5%, haplotype 3 (C-A) 11.9% and haplotype 4 (T-G) in only one allele of 2318 chromosomes. Genotype distributions were observed in Hardy–Weinberg equilibrium and similar between genders and across age categories. $Pvu$II-$Xba$I haplotype 1 was found associated with decreased plasma E2 levels in an allele-dose-dependent manner; per copy of haplotype 1 E2 levels were 1.68 pmol/l lower (P < 0.05). After adjusting for age, BMI, years since menopause and smoking, genotype-dependent differences increased slightly and plasma E2 levels decreased by 1.90 pmol/l per allele copy of haplotype 1 (P < 0.05). ESR1 haplotype 2 showed a similar significant, but opposite allele-dose effect on plasma E2 levels in women: per copy of haplotype 2 E2 levels were 1.74 pmol/l higher (P < 0.05). The results remained similar after adjustment for age, BMI, years since menopause, smoking and testosterone levels. ESR1 haplotype 3 was not associated with plasma E2 levels. None of the ESR1 haplotypes was associated with plasma testosterone.

Santana et al., (2006) performed ESR genotyping via PCR-RFLP from three purebred pig breeds: Large White (336 females and 26 males), Landrace (304 females and 27 males) and Pietrain (125 females and 11 males). For each breed, genotypes for the ESR gene were compared independently for expected progeny differences (EPD) in litter size (LS), average daily weight gain (DWG) (g/day) and back fat thickness (BT) as measured in mm by ultrasound. In the Large White breed, but not the other breeds, the ESR genotype was significantly (p < 0.05) associated to LS, DWG and BT. Large Whites genotyped as AA or AB had higher EPD values for the LS and BT traits compared to BB Large Whites, while AA Large Whites had higher DWG, EPD values than BB Large Whites. The A allele showed a beneficial effect on LS, DWG, BT and expected progeny differences for the Large White population. Due to the fixed allele A in Pietrain animals, analysis of variance and the mean contrast test were performed only for Landrace and Large White data. Although Landrace pigs genotyped as BB had higher EPD values than AA or AB animals for the LS, DWG and BT traits, the differences were not statistically significant (p > 0.05).
Zhen-Fang et al., (2006) studied the potential use of estrogen receptor gene (ESR) as a genetic marker for the selection of litter size in the Landrace population. ESR PCR product digestion by PvuII were determined in 2239 litters from 612 Landrace sows. The effects of ESR of different parities were not equal and the data of the first, second, and later parities were separately evaluated. The frequency of the B allele was much lower than that of the A allele. The frequency of allele A was 0.88 for the first parity data, and 0.87 for the second parity data. The values of likelihood ratios were 2.6012 and 3.5295, and single tail P values were 0.1068 and 0.060 for the first and second parity data, respectively. Both ratios were lower than the critical value of 3.84, i.e., the allele frequencies were in Hardy-Weinberg equilibrium. With regard to the traits of TNB (total number of piglet born) and NBA (number of piglets born alive), BB sows were superior to sows of the other two genotypes; AA and AB, which did not exhibit significant differences between them. The sows with the BB genotype showed better performance for TNB and NBA, but had a lower average piglet weight at birth (AWB). Piglet birth weights of sows of AA and AB genotypes were generally more than that of the BB genotype, especially for primiparous gilts.

Terman et al., (2006) determined the mutations in the ESR/AvaI (ESR1) and ESR/PvuII (ESR2) gene in 127 boars via PCR-RFLP kept at the AI Station and its effect on selected quantitative and qualitative characters of the semen. Two alleles of ESR1: A (109 and 76 bp) and B (76, 62 and 47 bp) along with three genotypes named AA, AB and BB and also two alleles of ESR2: C (120 bp) and D (65 and 55 bp) were identified with three genotypes CC, CD and DD with the frequencies: 0.79 (A), 0.21 (B), 0.83 (C) and 0.17 (D) respectively. In the studied population of boars, the genotype AA was detected with the frequency 0.69, AB-0.21, BB-0.10 of ESR1 and CC-0.72, CD-0.23, DD-0.05 of ESR2. The following lengths of restriction fragments were detected: 109 and 76 base pair (bp) for allele A, and 76, 62 and 47 bp for allele B. Whereas, the lengths of restriction fragments detected were: 120 bp for allele C and 65 and 55 bp for allele D. The analysis of relation between the genotype of estrogen receptor and the studied reproductive traits showed statistically significant differences (P < 0.01) between all semen traits of boars carrying different ESR1 and ESR2 genotypes.

Munoz et al., (2007) carried out a study to search for polymorphisms in the coding region of the estrogen receptors 1 and 2 (ESR1 and ESR2) and analyzed the effects of
these variants and the well known intronic ESR1 *Pvu*II polymorphism on litter size in a Chinese-European pig line. Five silent single nucleotide polymorphisms (SNP) in the ESR1 cDNA were identified: c.669T > C (exon 3), c.1227C > T (exon 5), c.1452C > T (exon 7), c.1665T > C and c.1755A > G (exon 8). One pair of these SNP (c.1665T > C and c.1755A > G) co-segregated in the analyzed line and the SNP c.669T > C showed the same segregation pattern as the *Pvu*II polymorphism. The ESR1 *Pvu*II and c.669T > C polymorphisms co-segregated in this line in the combinations [A; c.669T] and [B; c.669C]. The c.1665T > C and c.1755A > G ESR1 allelic variants co-segregated in the combinations c.[1665C; 1755G] and c.[1665T; 1755A]. No co-segregation was observed between the c.1227C > T or c.1452C > T polymorphisms within exons 5 and 7 respectively and other SNP identified in the ESR1 gene. No significant effects on litter size were found for the [c.1665C; c.1755G][c.1665C; c.1755G] and [c.1665C; c.1755G][c.1665T; c.1755A] genotypes. Nevertheless, the c.1227T allele was significantly associated with a higher total number of piglets born. The additive substitution effect was estimated to be 0.40 piglets per litter (P < 0.03), and no dominance effects were observed. Moreover, there was a consistent favorable effect of the c.1227T allele on NBA (0.25 piglets), without statistical significance (P < 0.14). In the ESR2 cDNA, one silent SNP was detected within exon 2 (c.486A > G) in samples from the Meishan breed and one missense SNP was found within exon 5, which caused an amino acid substitution in the coded protein: “c.949G > A (p.Val317Met)” and was tested on sow litter size. Information on 1622 litter records from 408 genotyped sows was analyzed to determine whether these SNP influenced the total number of piglets born (TNB) or the number of born alive (NBA). The polymorphisms ESR1: [*Pvu*II; c.669T > C], ESR1: [c.1665T > C; c.1755A > G] and ESR2: c.949G > A showed no statistically significant association with litter size.

Kjaergaard *et al.*, (2007) hypothesized that the estrogen receptor a (ESR1) IVS1-397T/C polymorphism affects high-density lipoprotein cholesterol response to hormone replacement therapy and risk of cardiovascular disease (CVD), cancer of reproductive organs, and hip fracture. They reported that the ESR1 IVS1-397T/C polymorphism did not influenced high-density lipoprotein cholesterol response to hormone replacement therapy or risk of CVD, most cancers of reproductive organs, or hip fracture.

Boekhoorn *et al.*, (2007) investigated the genetic variations in the estrogen receptor (*ESR1*) gene and the association of *ESR1* *Pvu*II-*Xba*I haplotypes with incident AMD.
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(Aging Macula Disorder) in a population of 4571 participants (aged 55 years and older). ESR1 PvuII-XbaI haplotype 1 was found to be a risk factor for late AMD. Correction for additional confounders, including age at menopause, use of hormone replacement therapy, blood pressure, and body mass index did not essentially alter the findings. ESR1 haplotype 1 was not found in association with any of the baseline characteristics, including age at menopause and use at any time of hormone replacement therapy. After an average follow-up time of 7.7 years (range, 0.3–13.9 years), 639 (14.0%) persons had iAMD, out of which 544 (11.9%) had early iAMD, and 95 (2.1%) had late iAMD, which further had 38 dry and 57 wet iAMD. The four possible PvuII XbaI haplotype alleles were observed in the following frequencies: haplotype 1 (T-A) 53.0%, haplotype 2 (C-G) 35.0% and haplotype 3 (C-A) 12.0%, whereas haplotype 4 (T-G) was absent in the studied population. Genotype and allele distributions were in Hardy-Weinberg equilibrium. After stratification by type of end stage, it seemed that particularly wet iAMD was responsible for this association. Individuals with one copy had a 3.72 (95% CI, 1.31–10.55) times higher risk of wet iAMD, whereas this was 4.71 (95% CI, 1.62–13.66) for individuals with two copies. Early iAMD was not associated with ESR1 haplotype 1. In conclusion the ESR1 PvuII XbaI haplotype 1 was found to be strongly associated with late iAMD.

Duss et al., (2007) created an ERa-positive breast cancer model and have forced normal HMECs (human mammary epithelial cells) derived from reduction mammoplasty tissue to express ERa in combination with other relevant breast cancer genes because ERa expression rapidly lost when normal HMECs were grown in vitro, breast cancer models derived from HMECs were ERa-negative. Candidate genes were selected based on breast cancer microarray data and cloned into lentiviral vectors. Primary HMECs prepared from reduction mammoplasty tissue were infected with lentiviral particles. Microarray analysis of ERa-positive tumours revealed that they commonly overexpressed the Polycomb-group gene BMI1. Lentiviral transduction with ERa, BMI1, TERT and MYC allowed primary HMECs to be expanded in vitro in an oestrogen dependent manner. Orthotopic xenografting of these cells into the mammary glands of NOD/SCID mice resulted in the formation of ERa-positive tumours that metastasised to multiple organs. The cells remained wild type for TP53, diploid and genetically stable. In vivo tumour growth and in vitro proliferation of cells explanted from tumours were found dependent on oestrogen.
Jakimiuk et al., (2007) evaluated ERα PvuII and XbaI polymorphisms by PCR-RFLP in population of Polish postmenopausal women. The absence of PvuII and XbaI restriction sites were indicated by "P" and "X" and presence by "p" and "x", respectively. PvuII genotype was found distributed as: PP 17.2% (n=11), Pp 50% (n=32), pp 32.83% (n=21) whereas frequency of XbaI genotype was: XX 6.25% (n=4), Xx 34.4% (n=22), xx 59.4% (n=38). Four haplotypes with following frequencies were recognized: PX 17.3%, px 47.4%, Px 24.4% and pX 10.9%. Prevalence of estrogen receptor alpha PvuII and XbaI polymorphisms in Polish women was similar to previously studied population. The distribution of genotypes was reported to in Hardy-Weinberg equilibrium. As expected, linkage disequilibrium between Pvu II and Xba I polymorphisms was also observed ($x^2=32.7$, df=2, $p<0.000001$).

Surekha et al., (2007) attempted to evaluate the role of ERα polymorphism in the breast cancer study and underwent a case control study to compare 250 breast cancer patients with 250 age matched healthy controls. The frequency distribution of Pvu II polymorphism in the ERα gene was assessed by PCR-RFLP method. The frequency of the PP genotype (35.3%) got elevated significantly in breast cancer patients when compared to controls (19.8%), with a corresponding increase in P allele frequency ($x^2=16.4$; $P=0.0003$). The OR (odd ratio) for genotypes PP Vs Pp was calculated to be 1.989 (95% CI: 1.2708 to 3.113). Premenopausal women with breast cancer had an elevated frequency of the PP genotype (22.8%) as compared to postmenopausal women (16.8%). When stage of disease was considered, both Pp and pp genotype frequencies were elevated in patients with advanced stage breast cancer. The frequency of the P allele and PP genotype frequencies tended to increase with increase in body mass index, whereas the Pp genotype frequency was elevated only in obese patients. The reverse was observed in in case of pp genotype frequency. The study thus highlighted the influence of ERα Pvu II polymorphism on the development and progression of breast cancer.

Humpolicek et al., (2007) designed to determine the associations between the Estrogen Receptor Gene (ESR - PvuII) and traits characterizing the sow efficiency comprehensively. Concretely, the age of the first conception (AFC), service period (SP), insemination interval (InI), total number of piglets born (TNB), number of piglets born alive (NBA), number of piglets weaned (NW), lean meat content (LMC), backfat thickness (BFT) and average daily gain (ADG) were also included in the study. Significant
effects of ESR gene on litter size (particularly in the second parity) and on reproduction traits were found. The ESR gene was also found to had significant effects on SP (P < 0.05) and InI (P < 0.01 and P < 0.001) in the 1st–6th parities. Sows with AB genotypes had demonstrably longer SP and InI than sows with AA genotypes. No significant effect of ESR gene on litter size in the 1st and 1st–6th parities was found. Reversely, in the 2nd parities, the ESR gene had an impact on the total number of piglets born, number of piglets born alive as well as on the number of piglets weaned.

Molvarec et al., (2007) analyzed blood samples from 119 severely preeclamptic patients and 103 normotensive, healthy pregnant Caucasian women using PCR-RFLP. Although, the PvuII and XbaI ESR1 genotype distributions were found in Hardy-Weinberg equilibrium in both groups, the two polymorphisms were only 50 bp apart in the first intron of the ESR1 gene. There were no significant differences between the groups in the genotype and allele frequencies of PvuII and XbaI polymorphisms. However, regarding the simultaneous carriage of both polymorphisms, the TT/AA genotype combination occurred significantly more frequently in the severely preeclamptic group (24.4% vs. 9.7%, p=0.003), whereas the frequency of the TT/AG genotype combination was significantly lower in that group (5.0% vs. 18.4%, p=0.002). Although the CC/GG genotype combination was more frequent in the severely preeclamptic patients, this association disappeared in a multivariate logistic regression model after adjustment for maternal age, prepregnancy BMI, primiparity and smoking status. According to the haplotype estimation, the homozygous T-A haplotype carriers had an increased risk of severe preeclampsia, independent of maternal age, prepregnancy BMI, primiparity and smoking status (adjusted odds ratio [OR]: 4.36, 95% confidence interval [CI]: 1.65–11.53). The risk of heterozygous T-A haplotype carriers did not differ from that of noncarriers (adjusted OR: 1.34, 95% CI: 0.64–2.83). Simultaneous carriage of the two polymorphisms and the PvuII polymorphism separately was not found to be associated with fetal growth restriction in severely preeclamptic patients. However, the GG genotype of the XbaI polymorphism was associated with a lower risk of fetal growth restriction in patients with severe preeclampsia (OR: 0.23, 95% CI: 0.07–0.73).

Tienthai et al., (2008) accessed the presence of ERα and PR (progesterone receptor) in the lining epithelium of Thai swamp buffalo oviduct during the follicular (n=12) and luteal phase (n=8), classified by the ovarian status. The results indicated that
the oviductal ER a and PR in Thai swamp buffalo oviduct varied according to the phase of ovarian activity and the regional function of the oviduct. Both immunoreactive ERa and PRB were present in the nuclei of the epithelial cells in all parts of Thai swamp buffalo oviduct and the staining was very clear in the lining epithelium of oviduct at the follicular phase. The intensities and proportion of both receptors were significantly higher in UTJ (uterotubal junction) and isthmus at the follicular phase compared with the luteal phase (P<0.05) while the significant differences in ampulla and infundibulum at both phases were not observed (p>0.05). The correlation of the present data with the previous report indicated that estrogen stimulated the expression of ERa and PR mRNA, whereas progesterone stimulation resulted in a reduction of both ERa and PR transcripts in the bovine oviduct. It was speculated that the presence of ERa and PR during the estrogen-dominated follicular phase might account for specific composition changes of the oviductal fluid, particularly in the sperm reservoir, i.e. UTJ and caudal isthmus of the ruminant.

Wedren et al., (2008) investigated the association of polymorphic variation in the estrogen receptor alpha gene (ESR1) with endometrial cancer risk. In 702 cases with invasive endometrial cancer and 1563 controls, five markers in ESR1 were genotyped and used logistic regression models to estimate odds ratios (OR) and 95 % confidence intervals (CI). An association between rs2234670, rs2234693, as well as rs9340799 markers was found to be in strong linkage disequilibrium (LD). The association with rs9340799 was the strongest, OR 0.75 (CI 0.60–0.93) for heterozygous and OR 0.53 (CI 0.37–0.77) for homozygous rare compared to those homozygous for the most common allele. The association between rs9340799 and endometrial cancer was not convincingly modified by exposures that alter the availability of estradiol or estrone such as use of menopausal hormone preparations or high BMI. The non-coding variation in ESR1 was also shown to be associated with endometrial cancer risk. With regard to the rs9340799 locus, homozygotes for the rare G allele had an almost halved risk for endometrial cancer compared to common allele homozygotes. No evidence of additional effects of markers other than rs9340799 was found. Haplotype models did not fit better to the data than single marker models. Further, genotype frequencies did not differ between those included in multivariate models and those excluded due to missing information about one or more of the included covariates.
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Pouresmaeili et al., (2009) assessed the relation between estrogen receptor-a gene polymorphism and osteoporosis in a population of 200 pre- and/or postmenopausal Iranian women (aged 35-80 years) stratified for BMD into normal and patient groups. Genetic polymorphism study and the identification of the related genotypes was carried out by restriction enzymes PvuII or XbaI. The genotypes of intron 1 PvuII or XbaI polymorphisms of the ER-a gene were detected and introduced so that the upper case and lower case letters of Pp (PvuII) and Xx (XbaI) signified the absence or presence of restriction sites in RFLP experiments. No significant relationship was observed between BMD and intron 1 RFLPs of the estrogen receptor alpha gene. Three genotypes, Pp XX, pp XX and PP xx, were detected, all at a very low frequency in this population of Iranian women. The difference between the two groups in spine BMD according to T-scores and Z-scores was found to be significant (p<0.0001). Interestingly, 25% of the patients showed osteoporosis while the control group showed no osteoporosis of the spine area (Tscore ≥-2.5) with the same evaluation, indicating a significant difference in the percentage of osteoporosis (p<0.0001).

Lu et al., (2009) analyzed the association of estrogen receptor a (OR a) gene polymorphisms with cytokine genes expression in patients with systemic lupus erythematosus (SLE) and controls. Genetic polymorphisms for XbaI (XX, Xx, or xx genotype) and PvuII (PP, Pp, or pp) in intron 1 of ORa gene were detected by polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP) method. The distribution of genotypes in both patients and controls was in agreement with the Hardy-Weinberg equilibrium (P > 0.05). There was no significant difference in the allele frequency of PvuII or XbaI RFLPs between control and SLE group. Genotypes ppXX and ppXx were not found in any participant and PpXX was not found in any SLE patient. PpXx, PpXx, and ppXx were found to be 3 major genotypes in patients with SLE and controls. Furthermore, the mRNA levels of interleukin (IL)-10, IL-4, interferon (IFN)-y, and IL-2 were assessed by semiquantitative reverse transcription polymerase chain reaction (RT-PCR). ORa gene polymorphism may be associated with the expression of IL-10, IL-4, IL-2, and IFN-y in patients with SLE. There was no significant difference in mRNA expression of 4 cytokines among controls with various genotypes. The level of IL-10 mRNA in patients with PpXx was higher than in patients with PPXX, PpXx, PpXX, and ppXx genotype (P = 0.018, P < 0.001, P < 0.001, P < 0.001, respectively), while the level
of IL-4 mRNA in patients with PpXx genotype was higher than in patients with PPXX, Ppxx, and ppxx (P = 0.027, P = 0.030, P = 0.021, respectively). The level of IFN-y mRNA in patients with PpXx genotype was lower than in patients with PPXX, PPXx, PPxx, PpXx, and ppxx (P = 0.009, P = 0.010, P = 0.046, P = 0.003, P < 0.001, respectively), while the level of IL-2 mRNA in patients with PpXx genotype was lower than in patients with PPXX, PPXx, PpXX, and ppxx (P = 0.035, P = 0.042, P = 0.001, P = 0.001, respectively, Mann-Whitney test).

Graeser et al., (2009) investigated the correlation of ER (estrogen receptor), PR (progesterone receptor) status, grading, age of onset and tumor size in 587 breast cancer patients with deleterious BRCA1 mutations. ERa and PR expression was found to got decreased from 62% in ductal carcinoma in situ (DCIS) to 20% and 16% in pT3 tumor, respectively (p value for ER 0.025 and PR 0.035, Fisher’s exact test). The percentage of grade 1/2 tumors decreased from 44% in DCIS to 17% in pT3 (p value 0.074). Thirteen women were diagnosed with DCIS, 321 women with pT1-size tumors, 228 women with pT2 tumor, and 25 women with pT3 tumor. The proportion of cancers that were ER a-positive decreased considerably from grade 1 to 3 (grade 1, 57.1% ER a positive; grade 2, 45.6%; and grade 3, 17.8% ER a positive; p value <0.001). A similar relationship was found for PR status and grading (grade 1, 42.9% PR positive; grade 2, 39.5%; and grade 3, 20.7% PR positive; p value <0.001). An increase was observed in ER a and PR positivity for tumors diagnosed under 30 years (24.6% ERa and 20.0% PR) to tumors diagnosed above 60 years (55.6% ER and 40.7% PR; ptrend=0.009 and ptrend=0.004, respectively). An inverse correlation between ER a and PR expression and grading was also detected which was in agreement with Atchley et al. (2008) who found that the proportion of tumors that were ER a-positive decreased as the histological grade increased.

Abbasi et al., (2009) conducted a study to establish a database of polymorphisms in Iranian population in order to compare Western and Iranian (Middle East) distributions and to evaluate ESR1 polymorphism as an indicator of clinical outcome. Allelic frequencies of exon 1 in the ESR1 gene among 297 Iranian women (150 breast cancer patients and 147 healthy control individuals) was screened for mutation or variant sites of single nucleotide polymorphisms (SNPs) via PCR-SSCP and DNA sequencing. The observed numbers of individuals with different genotypes showed that SNP fitted the Hardy-Weinberg equilibrium for both control and patient groups (p>0.05). Data suggested
that ESR1 polymorphisms were correlated with various aspects of breast cancer in Iranian ESR1 genotype, as determined during pre-surgical evaluation, might represent a surrogate marker for predicting breast cancer. The results also showed novel mutations but in the Iranian population studied it also revealed the presence of a silent common SNP rs2077647 (dbSNP128) in codon 10. The frequency of allele 1 in codon 10 (TCT---TCC) (T/C, S392S), was higher in cancer patients (about 50%) than in control individuals (about 40%); although the difference was not statistically significant (p = 0.148). The results demonstrated that codon 10 SNP might have protective action against breast cancer. The global geographical distributions of ER-a polymorphism in codon 10, reveals that exon 1 is significantly different in comparison with reported Western genomic studies. Further comparison showed that the frequency of allele 1 in codon 10 in Iran (46%) matches that in the USA (45%) and lower than in Australia (51%), higher than England (41%) and much greater than in Taiwan (32%).

Cupisti et al., (2009) investigated genetic variations in the estrogen pathway and their association with miscarriages in a total of 483 patients. Three variants of the CYP19A1 gene (rs10046, rs4646 and rs700519) and one variant each of the estrogen (ESR1) and progesterone (PGR) receptor genes (rs3020314 and rs1042838) were investigated using polymorphism genotyping. The chi-squared test and one-way analysis of variation (ANOVA) were used for statistical analysis. For rs10046 (CYP19A1), the C/C genotype was associated with a greater frequency of miscarriages (P = 0.017) whereas, the other genotypes were not found to be associated with recurrent miscarriage. SNP rs4646 was not consistent (P < 0.05). A single-nucleotide polymorphism in the aromatase gene (Aromatase is an essential enzyme in the estrogen pathway) was identified for the first time that suggested a significant association between genotypes and miscarriage. The minor allele frequency (MAF) for SNP rs700519 was 2.8%, but all other MAFs were larger than 18.2%. SNPs rs10046 and rs4646, which were located on the 3’-UTR region of CYP19A1, were strongly linked to each other (x² test, P < 0.001). Whereas none of the other genotypes had any associations with each other. Women with a T/T genotype experienced more than one miscarriage in 11.7% of cases, whereas for women with a C/C genotype or C/T genotype the figures changed to 3.3 and 4.3%, respectively (P = 0.017). For rs4646 (CYP19A1), the frequency of the wild type in women with recurrent miscarriages tended to be higher than for the heterozygous and variant type, but this divergence was not significant. Furthermore, no association was found between the
genotype frequencies of the rs3020314 (ESR) and rs1042838 (PGR) polymorphisms and the number of miscarriages.

Miller et al., (2010) attempted to determine the association of PMDD (Premenstrual Dysphoric Disorder) diagnosis related traits with SNPs in estrogen receptor alpha (ESR-1). Although PMDD being a heritable disorder, it was unclear whether any of the heritable susceptibility to PMDD resided in heritable personality traits. Seven out of 25 traits were observed to be significantly different in 68 patients and 56 controls as: Neuroticism (F=21.69, p<0.001), Extraversion (F=6.90, p<0.01), Emotional Stability (F=21.05, p<0.001), Abstractedness (F=7.62, p<0.01), Apprehension (F=8.35, p<0.005), Harm Avoidance (F=12.04, p<0.001) and Impression Management (used as an index of social desirability) (F=8.42, p<0.005). Genotypic associations revealed the following effects of the presence of a minor allele in patients and controls: lower Emotional Stability (L0058), lower Impression Management (L0058) (higher scores indicate a greater attempt to project a socially desirable image), higher Harm Avoidance (L0026, L0060, L0061), higher Neuroticism (L0026), and higher Abstractedness (L0025, L0026, L0060, L0061, L0055, L0058) (high scores reflect idea oriented, imaginative thinking while low scores reflect practical and concrete thinking) (F values 4.0 –9.0, p’s< 0.01–0.05). The results demonstrated affective state-independent personality traits that distinguished patients with PMDD from controls and further supported the relevance of ESR-1 polymorphic variants in the regulation of non-reproductive behaviours.

Zahmatkesh et al., (2011) investigated ER-a gene polymorphism in 5' region in consensus promoter for exon C along with A/G transition and determined its effect on production traits in 200 dairy Holstein using PCR-RFLP/ Bgl I technique and SAS software (Proc GLM). The fragment with 245 bp indicated allele A and two fragments with 168 and 77 bp represented allele G. So the digestion reaction resulted in two restriction fragments for GG, 3 fragments for AG and one uncut fragment for AA genotype. Total allelic frequencies were found to be 0.0742 and 0.9257 for allele A and G respectively and the total distribution of AA, AG and GG genotypes were 0.010, 0.129 and 0.861 respectively. The association study showed that ER-a genotypes had a significant effect on corrected milk fat (p<0.05) and milk fat percentage (p<0.05), as AG genotype increased fat yield and fat percentage in comparison with GG genotype. However AA genotype showed no significant difference with two other genotypes. No
relation of this SNP with milk production, corrected milk protein and protein percentage was found.

Szreder et al., (2011) studied the ER a A/C (RFLP-CfrI) genotypes in a cohort of 489 cattle including 355 Red and White cows and found a novel SNP using SSCP and DNA sequencing in the coding region of the estrogen receptor a (ERα) gene. DNA sequencing revealed the nucleotide substitution– A/C transversion at position 323,397 relative to the start of transcription site (Gen Bank, contig NW 932240.1), at position 1627 in the ERα mRNA (AY538775). The polymorphic site was located in exon 7, encoding the ligand-binding domain (LBD) of the receptor. After sequencing out of two patterns of SSCP, pattern 1 appeared the CC genotype while pattern 2 was identified as CA genotype while the AA homozygote was absent. Comparison of the restriction maps of ERα gene variants revealed that the C---A substitution created a new restriction site for CfrI. Thus, digestion with this endonuclease enabled PCR-RFLP analysis of the polymorphism. The nuclease cut the 266-bp DNA amplification product to 194 and 72 bp fragments for allele C, while allele A remains uncut. An association between ERα gene variants (A/C; RFLP–CfrI) and two dairy production traits (protein and fat content in milk) and sex of calf born was shown for the first time. No effects were detected of the ERα genotype on the reproduction traits studied.

Sato et al., (2011) investigated the possible influence of the single nucleotide polymorphism (SNP) of the estrogen receptor- P gene, rs1256049 (Rsa) in exon 5, on the frequency of rheumatoid arthritis (RA) in 263 RA patients and 174 control with osteoarthritis (OA) via PCR–RFLP method. The occurrence of both mutant allele (G) and genotype (GG) were significantly higher in RA than in OA patients (allele P = 0.008, OR: 1.501, 95%CI: 1.12–2.02). In RA patients, GG genotype frequency was found to be higher in severe RA patients than mild RA patients. Moreover, there was a significant difference between severe RA patients and OA patients (P = 0.009), also the allele distribution was considerably different between severe RA, mild RA, and OA patients (P = 0.025, 95%CI = 0.61–0.93). With respect to gender, GG genotype was statistically more frequent in female RA patients than that of OA, while such an association was absent in men. Above all, the presence of the GG genotype could be a risk factor for RA and such trend might be different in gender, although additional larger scale study is needed.
Wu et al., (2011) characterized the molecular actions of endoxifen in breast cancer cells expressing ERP and examined its effectiveness as an anti-estrogenic agent in the cell lines. MCF7, Hs578T and U2OS cells were transfected stably with full-length ERP. ERP protein stability, dimer formation with ERα and expression of known ER target genes were characterized followed by endoxifen exposure. The ability of various endoxifen concentrations was also determined to block estrogen-induced proliferation of MCF7 parental and ERP-expressing cells. The global gene expression profiles of these two cell lines was monitored following estrogen and endoxifen exposure and biological pathway analysis of these data sets was conducted to identify altered cellular processes. The endoxifen was showed to stabilize ERP protein, unlike its targeted degradation of ERα, and induced ERα/ERP hetero-dimerization in a concentration dependent manner. Endoxifen was showed to be a more potent inhibitor of estrogen target genes when ERP was expressed. Additionally, low concentrations of endoxifen observed in tamoxifen treated patients with deficient CYP2D6 activity (20 to 40 nM) markedly inhibited estrogen induced cell proliferation rates in the presence of ERP, whereas much higher endoxifen concentrations were needed when ERP was absent. Microarray analyses revealed substantial differences in the global gene expression profiles induced by endoxifen at low concentrations (40 nM) when comparing MCF7 cells which express ERP to those that do not. These profiles implicated pathways related to cell proliferation and apoptosis in mediating endoxifen effectiveness at these lower concentrations. Taken together, these data demonstrated the enhancement of sensitivity of breast cancer cells to the anti-estrogenic effects of endoxifen in presence of ERP. These findings suggested the need to further elucidate the role of ERP in the biology and treatment of breast cancer and suggested that the importance of pharmacologic variation in endoxifen concentrations might differ according to ERP expression.

Silva et al., (2011) examined the frequency of Rsal polymorphism of the ER P gene in patients diagnosed with endometriosis (grouped into fertile and infertile) and control persons. The genotype frequencies found in patients with endometriosis (N = 54) were 0% of the AA genotype, 59.3% of the AG genotype and 40.7% of the GG genotype. Among the control patients (N = 46) the frequencies were 0% of the AA genotype, 6.5% of the AG genotype and 93.5% of the GG genotype. The AG allele frequency in the fertile patients was approximately 10.5 times higher than in the control group, and the allelic frequency of the AG genotype in the infertile patients was 8.5 times higher compared to
controls (P < 0.0001 for both). The genotypic frequency of p53 and Rsal polymorphisms of the ER P gene in patients was 15/20 AG genotype of Rsal polymorphism of the ER P gene with Arg/Arg and 5/20 GG genotype for the same allele. The frequency of the AG genotype of the Rsal polymorphism of the ER P gene in the group with endometriosis was three times higher for the allele Arg/Arg than the GG genotype for the same allele.

Lurie et al., (2011) examined the association of ovarian carcinoma risk with the polymorphism rs1271572 in the estrogen receptor beta (ESR2) gene in 4946 women with primary invasive ovarian carcinoma and 6582 controls (Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using unconditional logistic regression adjusted for site and age. In all studies combined, women with the TT genotype had 35% increased ovarian carcinoma risk [odds ratio (OR) =1.10; 95% confidence interval (CI): 1.01–1.21; p= 0.04] compared to carriers of any G allele (recessive genetic model). In addition to the HAW study (Hawaiian Ovarian Cancer Study), the association was also statistically significant among AUS study (Australian National Ovarian Cancer Study) participants (OR = 1.25; 95% CI: 1.01–1.54; p =0.04). A significant interaction was observed between genotype and age (p = 0.02). The association of the TT genotype with risk among women <50 years old was also statistically significant in two individual studies, BAV (Bavarian Ovarian Cancer Cases and Controls) (OR = 3.64; CI: 1.35–9.83; p = 0.01) and MAL (The Danish Malignant Ovarian Tumor Study) (OR = 2.05; CI: 1.07–3.91; p = 0.03) and in the pooled analysis when HAW data were excluded (OR= 1.34; 95% CI: 1.11–1.61; p= 0.009). The association of the TT genotype with risk was also higher in premenopausal (OR =1.20; CI: 0.99–1.45; p= 0.06) compared to postmenopausal women (OR = 1.10; CI: 0.98–1.24; p = 0.12), although the test for heterogeneity in effect was not significant (p = 0.82).

Yan et al., (2012) examined the relationship between ESR1 polymorphisms with HBV (hepatitis B virus)-related acute liver failure (ALF) risk among chronic HBV carriers in a Chinese population (a total of 1216 unrelated Han Chinese HBV carriers including 359 HBV surface antigen (HBsAg) carriers affected with ALF and 857 asymptomatic HBsAg carriers). Two ESR1 haplotype were genotyped tagging polymorphisms, c.30 T>C (rs2077647) and c.453-397 T>C (rs2234693), via PCR-RFLP assay. A significantly increased susceptibility to HBV-ALF was observed associated with the c.30 C allele, c.453-397 C allele and [c.30 C; c.453-397 C] haplotype compared with the T alleles and
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The haplotype of c.30 T>C and c.453-397 T>C polymorphisms, respectively. This further suggested [c.30 C; c.453-397 C] haplotype to be a risk factor for genetic susceptibility to HBV-related ALF in the Chinese population and emphasised the importance of ESR1 in the pathophysiology of HBV-related ALF on the population level. The genotype distributions for the two SNPs were found in Hardy–Weinberg equilibrium in each group. Logistic regression analysis with adjustment for age, sex, HbeAg status and alcohol consumption indicated significant differences in the distribution of the three haplotype groups between the ASC and HBV-ALF groups.

Abbasi et al., (2012) studied the correlation of ER-a and ER-P genes with an additively increased risk for breast cancer in Iranian women by PCR single-strand conformation polymorphism (SSCP) method. Three single nucleotide polymorphisms (SNPs) in codon10 (TCT---TCC), codon 352 (CCG---CCC) and codon 594 (ACG---ACA) in ER-a gene and one SNP codon 392 (CTC---CTG) in ER-P were revealed to had additive effects in developing breast cancer and LN metastases. However, SNP in codon 392 of estrogen receptor-P gene was found to be more effective (threefold) than those SNPs in codons 10, 352, 594 of estrogen receptor-a gene in developing LN metastasis in breast cancer patients. In consideration of the age at menarche at 12- years- old (and above or below) in the different gene separately and together, patients with all three polymorphisms in ER-a gene revealed higher frequency i.e. 61.9% in comparison with the normal genotype with 25.8% (P=0.001). However, addition of ER-P polymorphism in codon 392 (CTC---CTG) rs1256054, did not showed additive effect in developing breast cancer (54.5%). It was further demonstrated that a combination of the three SNP markers may increase accuracy in predicting development of breast cancer for individuals with age at menarche below 12 years old.

Xin et al., (2012) explored a potential association between the estrogen receptor P (ER P) gene polymorphisms and vascular dementia (VaD) in women using unconditional logistic regression. The relationship of two polymorphisms (rs944050 and rs4986938) and their associated haplotypes in the ER P gene with VaD were examined in 121 Chinese Han women (>50 years of age) including 61 with VaD and 60 healthy age-matched controls. The variant allele G of rs944050 in the ER P gene increased the risk of VaD (odds ratio = 2.02, 95% confidence interval = 1.08-3.77). In haplotype analyses, the ER P haplotype containing the polymorphism rs944050 variant allele and the polymorphism rs4986938
wild-type allele was associated with VaD (odds ratio = 1.70, 95% confidence interval = 1.03-2.84). The polymorphism rs944050 in the ER P gene was further associated with an increased risk of VaD in Chinese Han women.

Renaville et al., (2012) investigated the polymorphisms in the estrogen receptor 1 (ESR1 PvuII 5700/4200) and estrogen receptor 2 (ESR2 A949G) genes in 612 pigs (278 females, 334 castrated males) and collected the following phenotypical data: carcass weight, lean percentage, leg weight, back fat and leg fat thickness. Castrated males of the ESR1 PvuII 5700/5700 genotype had significantly more back fat (P<0.05) with no significant effect on leg fat. Conversely, ESR1 PvuII 5700/5700 females had significantly less leg fat (P<0.05) with no significant effect on back fat. Both males and females of the ESR2 A949A genotype had less leg fat (P<0.05) without any effect of the polymorphism on back fat. The results also suggested that ESR1 PvuII 5700/4200 and ESR2 A949G polymorphisms were found associated with subcutaneous fat localization in pigs.

Wang et al., (2012) explored a more robust estimate of the role of estrogen receptor-a polymorphisms in endometrial carcinogenesis. The meta-analysis included 1944 cases and 3075 controls for PvuII polymorphisms and 1831 cases and 2875 controls for XbaI polymorphisms. The results did not showed any statistically significant association between endometrial cancer risk and either PvuII or XbaI polymorphisms. However, in case of PvuII polymorphisms, both the CT and the CC genotypes significantly increased the risk of endometrial cancer in an Asian-Australian population (pooled OR = 1.50, 95%CI 1.09-2.08 and pooled OR = 1.55, 95%CI 1.08-2.22, respectively). The CC genotype significantly decreased the risk of endometrial cancer (pooled odds ratio 0.77, 95% confidence interval 0.64-0.94) in a European population. In contrast, the stratified analysis suggested that the XbaI polymorphism was not significantly associated with endometrial cancer between different populations. This meta-analysis provided evidence that PvuII polymorphisms in the ESR1 gene, which encoded for estrogen receptor-a, could modify the susceptibility to endometrial cancer. However, the genetic effect varied between different geographical regions for unknown reasons.

Joanne et al., (2012) examined the association between estrogen receptor polymorphisms and depression-related mood disorders across the lifetime. The conducted studies produced inconsistent findings, which were likely related to the large heterogeneity in terms of the populations, study design and depression measures used. It appeared
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unlikely that the common ESR1 variants rs2234693 and rs9340799 were associated with moderate depressive symptoms in women; however, there was some evidence that indicated a significant association with more severe depressive symptoms, major depressive disorder and anxiety. A very few studies of ESR2 polymorphisms were also reported to draw any definite conclusions; however, preliminary evidence suggested that specific variants may modify the risk of depression associated with the use of hormone treatment in women. Few studies had also investigated associations in men and they focused almost exclusively on ESR1, but all reported non-significant findings. If it was possible to utilize estrogen receptor polymorphisms in association with the risk of depression, this could be important preventive and therapeutic implications. Such therapies might prove to be more effective in treating particular people with depression based on their genetic profile, which being an exciting prospect given that many people did not responded to current antidepressant treatments.

Janusz et al., (2014) investigated associations of the ESR1 and ESR2 polymorphisms with AAM (age at menarche) in IS (Idiopathic Scoliosis) patients and also evaluated the association of AAM with IS severity. The age at menarche is determined by both genetic and environmental factors as well as their interactions. Estrogen receptors 1 and 2 polymorphism were reported to be associated with AAM in ESR1 XbaI and PvulII site polymorphism and in ESR2 AluI site polymorphism. PCR-RFLP revealed four SNPs: XbaI (A/G rs9340799) and PvulII (C/T rs2234693) in ESR1 and AluI (A/G rs4986938) and RsaI (A/G rs1256049) in ESR2. All genotypes were in HWE (Hardy-Weinberg Equilibrium). Mean AAM for patients was calculated to be 154.8 ± 14.7 months (12.9 ± 1.2 years). No statistically significant difference was observed between AAM mean values in each genotype, for the XbaI, PvulII, AluI and RsaI site polymorphisms (p = 0.7141, 0.9774, 0.7973 and 0.2282 respectively). The Cobb angle was measured and the patients were divided into mild (<30°), moderate (30°-49°) or severe (≥50°) IS revealed tendency to delay AAM: 151.9 ± 14.7; 155.2 ± 14.8 and 157.9 ± 14.0 months, respectively. A statistical significant difference was found between patients with mild <30° and severe ≥50° IS (p = 0.0267).

Mahdavipour et al., (2014) investigated an association between estrogen receptor alpha (ESR1) gene polymorphisms and RPL (Recurrent pregnancy loss) in Iranian women with RPL. The study involved 244 women (with a history of three or more consecutive
pregnancy losses) and 104 healthy women (with at least two live births). PCR-RFLP method was used to study -397C/T and -351A/G polymorphisms on ESR1 gene in both the case and control subjects. The genotypic frequencies of -397C/T and -351A/G polymorphisms on ESR1 did not differ significantly between RPL and control groups (p=0.20 and p=0.09, respectively). A significantly negative correlation was observed between -397C/T and -351A/G (r=-0.852, p<0.001) in RPL women and complete linkage disequilibrium was found between the investigated polymorphisms (D’: 0.959; r-square=0.758, p<0.001). It was hence suggested that the analyzed polymorphisms on ESR1 gene were not associated with an increased risk of RPL in the studied population.

2.2 Molecular characterization of progesterone receptor gene

Progesterone is a steroid hormone produced mainly by the ovaries, and functions as essential regulator of normal female reproductive system in the uterus, ovary, mammary gland and brain. It also plays an important role in non reproductive tissues such as cardiovascular system, bone and central nervous system, highlighting the widespread role of this hormone in normal physiology (Graham and Clarke, 1997; Graham and Clarke, 2002; Li et al., 2004; Mote et al., 2007). Progesterone is mandatory requirement for uterine implantation, decidualization, maturation of mammary epithelium and maintenance of pregnancy (Li et al., 2004).

Major target tissues of progesterone includes those present in the reproductive tract such as the ovary, uterus and vagina as well as the mammary gland, pituitary and hypothalamus (Kimbrel and Mcdonnell, 2003). The effects of progesterone are triggered after binding of progesterone to its intracellular receptor, the progesterone receptor (PGR). PGR was discovered in 1970 as affinity binding partner for progesterone with the corresponding gene locating to the long arm of chromosome 11(11q22.1) (Ellmann et al., 2009). Progesterone receptors are members of a large family of structurally related gene products known as the nuclear receptor (NR) super family. In humans, primates, pigs, rodent, and chicken, nuclear PGR consist of 2 distinct isoforms, PGR-A and PGR-B, derived from a single gene independently regulated by separate promoters. Importantly, the two PGR isoforms are not functionally equivalent, and in vitro experiments show that PGRA functions as a transcriptional inhibitor of PGR-B when PGR-A and PGR-B are co-expressed (Conneely et al., 1989, Kastner et al., 1990). The production of these two isoforms is conserved in a number of vertebrate species including humans and rodents and
the ratios of the individual isoforms vary in reproductive tissues as a consequence of developmental and hormonal status and also during carcinogenesis (Evans, 1988; O’Malley & Conneely, 1992 and Mangelsdorf et al., 1995).

PGR is composed of a variable N-terminal transactivation domain, a highly conserved DNA-binding domain, a hinge domain, and a ligand-binding domain. In the absence of an activated ligand, PGR is inactivated through association with various heat shock proteins (hsp), including hsp90, hsp70, and hsp40, and other co-repressor proteins. Ligand binding causes release of the multiple hsp-subunit complex and the PGR undergoes a conformational change that allows the receptor dimer to interact with specific progesterone response elements located within the regulatory regions of its target genes (Li et al., 2014). The PGR isoforms are highly similar except that the PGR-B isoform exhibits an extra 164 amino acids at the amino terminus. Within this sequence resides an extra activation domain known as AF-3 which bestows unique functions to the PGR-B isoform (Sartorius et al., 1994). The PGR-A isoform was identified to exhibit a trans-dominant repressive role on gene transcription, while PGR-B often promoted the transcription of genes (Vegeto et al., 1993). The mechanism of inhibition of the inhibitory domain by the AF-3 domain was identified as a single phosphorylated serine residing within the AF-1 domain (Clemm et al., 2000). Therefore, the presence of the AF-3 domain proved to be responsible for the positive transcriptional activity of the PGR-B isoform. Within the mouse, the PGR isoforms have also proven to display distinct functions. Mulac-Jericevic et al., 2000 generated mice with selective ablation of the PGR-A isoform, the mouse resembled the PRKO mouse, displaying infertility with defects in uterine and ovarian function. However, the PGR-B ablation mouse model was found dispensable for uterine function, yet necessary for normal mammary development and branching (Mulac-Jericevic et al., 2003). Therefore, although highly similar, the PGR isoforms have demonstrated to be responsible for different functions within the murine reproductive system (Wetendorf and Demayo, 2014).

Conneely et al., (1989) studied the chicken progesterone receptor A and B proteins and found that they differed by an additional 128 amino acids located at the N terminus of the B protein. Using site-directed mutagenesis, it was demonstrated that these two proteins formed by alternate initiation of translation from two in-phase AUG codons located on a single mRNA transcript. Both proteins were transcriptionally active in terms of their
ability to activate a progesterone-responsive target gene. A functional significance was further suggested for evolutionary conservation of two forms of this steroid receptor.

De Vivo et al., (2002) genotyped all 187 documented cases of invasive endometrial cancer from 1978 to 1996 and 397 matched controls for all of the polymorphisms and stated that the excessive estrogen stimulation unopposed by progesterone may got strongly predisposed to endometrial cancer. As the progesterone receptor (PR), existing in two isoforms, PR-A and –B; is required for the anti proliferative effect of progesterone, the variants in the PR gene may might got predisposed to endometrial cancer. Six variable sites were found including four polymorphisms in the hPR gene and five common haplotypes. One promoter region polymorphism, +331G/A created a unique transcription start site. Biochemical assays showed that the +331G/A polymorphism increased the transcription of the PR gene, favouring production of hPR-B in an endometrial cancer cell line. In a case control study, a statistically significant association was observed between the +331G/A polymorphism and the risk of endometrial cancer, which was found even greater in overweight women carriers. These associations remained unchanged after including a second population of controls for the analysis. Hence, the +331G/A hPR gene polymorphism was suggested to contribute to endometrial cancer risk by increasing expression of the hPR-B isoform.

Mulac-Jericevic et al., (2003) generated a mouse model in which expression of PR-B was specifically ablated (PRBKO−/−) to address the physiological role of PR-A. The selective activation of PR-A in PRBKO−/− mice was shown sufficient to elicit normal ovarian and uterine responses to P (Progesterone) but resulted in reduced mammary gland morphogenesis. In the absence of PR-B, pregnancy-associated ductal side branching and lobulo alveolar development were reduced markedly due to decreased ductal and alveolar epithelial cell proliferation and decreased survival of alveolar epithelium. In an effort to elucidate the molecular genetic signaling pathways that were differentially regulated by PRs in the mammary gland, receptor activator of nuclear factor kB ligand (RANKL) was identified as a paracrine mediator of P-dependent alveologenesis. Further, it was demonstrated that the defects in PRBKO−/− mice were associated with an inability of PR-A to activate the RANKL signaling pathway in response to P. The functional interaction between PR-A and PR-B was not supposed to be required for reproductive activity and the
selective modulation of PR-A activity by progestin agonists might have a protective effect against both uterine and mammary gland hyperplasias.

De Vivo et al., (2003) suggested an important role of progesterone in mammary tumorigenesis and also determined whether or not the functional polymorphism, +331 G/A, which caused an increase in the expression of the hPR-B isoform, was related to breast cancer risk. Using a nested case-control study, 990 cases and 1,364 controls were genotyped and observed a statistically significant increased risk of breast cancer among carriers of the +331 A allele (odds ratio, 1.33; 95% confidence interval, 1.01–1.74) compared with subjects with the GG genotype. The genotype distribution of the +331 G/A polymorphism among the cases and controls was in Hardy-Weinberg equilibrium (P = 0.11). A statistically significant increased risk of breast cancer was observed among carriers of the +331 G/A polymorphism; compared with the +331 G/G wild-type genotype, the adjusted OR for women with +331 G/A and +331 A/A was 1.33 (95% CI, 1.01–1.74). After stratifying by menopausal status, a similar association was found among postmenopausal women (adjusted OR, 1.41; 95% CI, 1.06 –1.87) but not among premenopausal women. The +331 G/A polymorphism was revealed to modify the association between BMI and endometrial cancer risk, a hormonally related cancer, and obesity was directly related to breast cancer only among postmenopausal women. It was also observed a potential interaction between genotype and body mass index (BMI) among postmenopausal women, with the highest risk (odds ratio, 2.30; 95% confidence interval, 1.02–5.21) among obese women (BMI >30 kg/m²) with the GA or AA genotype as compared to that of lean (BMI <25 kg/m²) women with the GG genotype. It was further suggested that the increased production of hPR-B by the +331 G/A polymorphism may predispose women to breast cancer development through increased hPR-B-dependent stimulation of mammary cell growth.

Berchuck et al., (2004) studied the effect of a functional SNP in promoter of progesterone receptor (+331 A) on ovarian cancer risk. The +331G/A polymorphism was genotyped in a population-based, case-control study from North Carolina including 942 Caucasian subjects (438 cases, 504 controls) and in a confirmatory group from Australia (535 cases, 298 controls). The observed SNP might have altered the relative abundance of the A and B isoforms and has been associated with an increased risk of endometrial and breast cancer. Examination of genotype frequencies by histologic type revealed that this
was due to a decreased risk of endometrioid and clear cell cancers (OR, 0.30; 95% CI, 0.09-0.97). Similarly, in the Australian study, there was a nonsignificant decrease in the risk of ovarian cancer among those with the +331A allele (OR, 0.83; 95% CI, 0.51-1.35) that was strongest in the endometrioid/clear cell group (OR, 0.60; 95% CI, 0.24-1.44). The +331A allele was found in 59 of 504 (11.7%) Caucasian controls and the distribution of genotypes was in Hardy-Weinberg equilibrium (P = 0.53). Among individuals who reported their race to be African American, only 1 of 81 (1.2%) controls and 0 of 67 with ovarian cancer carried the +331A allele. In view of the rarity of the +331A allele in African Americans, these subjects were excluded from analyses of the association with ovarian cancer risk. However, samples from the Australian study were also genotyped independently and 10.7% of controls were found to carry the +331A allele. Logistic regression analysis was used to calculate age-adjusted odds ratios (OR). Further, a protective effect of the +331A allele (AA or GA) against ovarian cancer was also suggested in the North Carolina study [OR, 0.72; 95% confidence interval (95% CI), 0.47-1.10]. In the combined U.S.-Australian data that included 174 endometrioid/clear cell cases (166 invasive, 8 borderline), the +331A allele was significantly associated with protection against this subset of ovarian cancers (OR, 0.46; 95% CI, 0.23-0.92). These findings suggested that the +331G/A progesterone receptor promoter polymorphism might modify the molecular epidemiologic pathway that encompasses both the development of endometriosis and its subsequent transformation into endometrioid/clear cell ovarian cancer. Although not statistically significant, a similar inverse association with invasive ovarian cancer risk was observed (OR, 0.83; 95% CI, 0.51-1.35).

Lisa et al., (2004) addressed the significance of phosphorylation of human progesterone receptors (PR) by cyclin-dependent protein kinase 2 (CDK2) at multiple sites, including Ser400. The regulated phosphorylation of Ser400 in response to progestins and mitogens was also revealed by PR phospho-Ser400-specific antibodies and was correlated with increased CDK2 levels and activity. Expression of cyclin E was found to elevate CDK2 activity and down regulate the PR independently of ligand. Similarly, over expression of activated mutant CDK2 increased PR transcriptional activity in the absence and presence of progestin. Further, the mutation of PR Ser400 to alanine (S400A) blocked CDK2-induced PR activity in the absence of progestin, but not in its presence. PR was observed to be unresponsive to activated CDK2 in breast cancer cells with elevated p27 and RNA interference knockdown of p27 partially restored CDK2-induced ligand-
independent PR activation. Similarly, it was observed that in p27\(^{-/}\) mouse embryonic fibroblasts, elevated CDK2 activity increased wild-type (wt) but not S400A PR transcriptional activity in the absence of progestin. CDK2 induced nuclear localization of unliganded wt but not S400A PR; liganded S400A PR exhibited delayed nuclear accumulation. These studies demonstrated that CDK2 regulate PR in the absence of progestins via phosphorylation of Ser400, thus revealing a novel mechanism for upregulated PR transcriptional activity in human breast cancer cells expressing altered cell cycle regulatory molecules.

De vivo et al., (2004) genotyped 1252 cases and 1660 matched controls with the use of the Taqman assay and reported that the PROGINS allele, which was in complete linkage disequilibrium with a missense substitution in exon 4 (G/T, valine\(\rightarrow\)leucine, at codon 660), was associated with a decreased risk for breast cancer in a predominantly Caucasian population (89% of cases, 86% of controls). The study was stratified by menopausal status and observed no association among premenopausal women (adjusted OR 1.21 [95% CI 0.64–2.28]) although a relatively small number of women were used for this analysis. Furthermore, an association between the Val660\(\rightarrow\)Leu PROGINS polymorphism and breast cancer risk was also not supported. However, the Val660\(\rightarrow\)Leu polymorphism has been suggested to modify the association between family history of breast cancer and breast cancer. In addition, BMI, history of benign breast disease and hormone replacement therapy use among postmenopausal women were selected as potential effect modifiers based on biological plausibility and no significant interactions were observed between the Val660\(\rightarrow\)Leu polymorphism and any of these risk factors. Cases and controls had similar mean BMI at blood draw (25.5 versus 25.5 kg/m\(^2\)) and weight gain since age 18 years (11.6 versus 11.3 kg). In comparison with controls, cases had similar ages at menarche (12.5 versus 12.6 years), first birth (23.0 versus 23.0 years) and age at menopause (48.2 versus 47.9 years). The proportion of women with a first-degree family history of breast cancer was significantly higher among the cases (20.0% versus 15.0%). Also the cases were more likely to have a history of benign breast disease (64.0% versus 51.0%) and a longer duration of postmenopausal hormone use (50.3% versus 49.7% current users for five or more years).

Terry et al., (2005) genotyped four polymorphisms in the progesterone receptor gene (+44C/T, +331G/A, G393G, V660L) and inferred haplotypes in 987 ovarian cancer
cases and 1,034 controls living in New Hampshire and Eastern Massachusetts. Odds ratios and 95% confidence intervals were calculated to evaluate associations with ovarian cancer. Five haplotypes occurred with greater than 5% frequency and the haplotype carrying the V660L variant had a significant association with ovarian cancer (odds ratio = 0.76, 95% confidence interval: 0.62, 0.92). Among Caucasian cases, the prevalence of variant carriers was 13% for +44C/T, 10% for +331G/A, 55% for G393G, and 28% for V660L compared with 13%, 10%, 53%, and 35%, respectively, for Caucasian controls. To understand the degree to which these polymorphisms were linked, standardized pairwise linkage disequilibrium tests were performed and showed that the linkage disequilibrium among +44C/T, +331G/A, G393G, and V660L ranged from 0.07 to 0.42.

After adjusting for potential confounders, no association was observed between variant carriers of +44C/T (odds ratio (OR) = 1.09, 95% confidence interval (CI): 0.82, 1.44), +331G/A (OR = 0.98, 95% CI: 0.71, 1.34), or G393G (OR = 1.12, 95% CI: 0.93, 1.34) and ovarian cancer risk. However, a statistically significant inverse association was observed between V660L (OR = 0.70, 95 percent CI: 0.57, 0.85) and ovarian cancer risk. The inverse association between V660L and ovarian cancer remained consistent across all ovarian cancer histologies, but only the associations with serous invasive (OR = 0.65, 95 percent CI: 0.49, 0.86) and endometrioid (OR = 0.66, 95 percent CI: 0.43, 0.99) histologies were significant.

Peiro et al., (2006) carried out genotyping of the SNP G/A2464 using PCR-RFLP among 494 female individuals, out of which a total of 171 animals GG, 247 AG and 78 AA were found in the F2 population. The GG genotype had around 0.6 implanted embryos (relevant difference) more than AA genotype with a high probability (P(D>0) 89%) and the probability of a relevant difference was moderate (P(D>b) was 60%) which was always in favour of GG genotype. This difference was around 0.7 kits and the probability of a relevant difference was high (P (D>b) is 79%) and always in favour of GG genotype. Further, the GG genotype had a higher ES (embryo survival) and FS (foetal survival) than AA genotype, P (D>0%) was 84% and 73%, respectively. Although these differences between genotypes were not relevant, around 3% in each survival trait, the probability of a relevant difference in favour of GG genotype were 39 and 43% respectively. The GG genotype had a higher PS ((D=4.6%); P (D>0) is 83%). Although HPD (highest posterior region) 95% included zero, the probability of a relevant difference was moderate (P (D>b) is 59%) and the probability of relevant difference in favour of GG genotype was 55%. The
genotype of the receptor of progesterone represented the 35% and 33% of the difference between H and L line found by Santacreu et al., (2005) for implanted embryos and number of total born. The polymorphisms analyzed in progesterone receptor that were associated to H and L lines could explain a large part of the difference found for implanted embryos (EI), total number of kits born (LS) and number of kits born alive (NBA).

Romano et al., (2007) assessed the prevalence, gene-gene and phenotype-genotype associations of two functional progesterone receptor polymorphisms (PRP), PROGINS and +331G/A, in familial BC (breast cancer) / OC (ovarian cancer). Over 95% of the families (204/214) presented at least one case of BC (maximum 6 cases; average age at diagnose: 51.1±9.9) and about half of the families (109/214) had three or more relatives with BC. Twenty-nine families (0.7%) presented at least one patient with two primary breast cancers. About one quarter of the families (51/214, 23.8%) had at least one case of OC and 19 families (9.0%) had two or more cases of OC (maximum 4 cases; average age at diagnose: 53.7±13.6). A total of 78 relatives having OC were recorded. BRCA1 was found to be associated with BC at young age, p=0.002; +331A marginally with OC, p=0.07, and PROGINS with male BC, p=0.04. Homozygous +331A/A co-segregated with BRCA2 variants more frequently than expected by chance alone. Co-occurrence of +331A with a BRCA1BRCA2 mutation was associated with multiple BC events compared to +331A or BRCA1/BRCA2 alone, p=0.02. The +331A allele was recognized as a gene modifier of BRCA1 and BRCA2.

Dassena et al., (2007) performed genomic profiling on explants of late proliferative phase human endometrium (after 24-h treatment with progesterone (P) or oestradiol and progesterone (17P-E2+P)) and on explants of menstrual phase endometrium treated with 17P-E2+P. The results showed that late proliferative phase human endometrium was more responsive to progestins than menstrual phase endometrium and the expression of several genes associated with embryo implantation (i.e. thrombomodulin, monoamine oxidase A, SPARC-like 1) could be induced by P in vitro and that the genes that were fully dependent on the continuous presence of 17P-E2 during P exposure could be distinguished from those that were P-dependent to a lesser extent. Therefore, 17P-E2 selectively primed implantation-related genes for the effects of P.

Karadeniz et al., (2007) studied 95 young women with PCOS (polycystic ovary syndrome) and 99 healthy control women and underwent complete hormonal assays, lipid
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PCOS patients were divided into three sub-groups (T1/T1, T1/T2, T2/T2) according to their PROGINS gene polymorphism. In the patients group no statistically significant differences in HDL – cholesterol, LDL – cholesterol, total cholesterol, triglyceride, fasting blood glucose, fasting serum insulin, HOMA-IR, prolactine, f-testosterone, total testosterone, estradiol, DHEAS, fibrinogen, hs-CRP and oxidative stress parameters was detected (p > 0.05). But a statistically significant difference was detected on serum FSH levels for T1/T1 genotype according to T2/T2 genotype. In PROGINS polymorphism results; in both control and the patient groups T1/T1 was detected in high levels. No relation was detected between the inflammatory and oxidative stress factors and PROGINS polymorphism alleles. This was probably because the PCOS patients were young and their BMI (body mass index) means were normal and their CIMT (Carotid intima-media thickness) and oxidative stress markers were like healthy women. As a result of this study; no difference was detected on different genotypes of functional progesterone receptor polymorphism (PROGINS) according to hs-CRP and fibrinogen proinflammatory markers, which were important factors for early atherosclerosis in PCOS. Also, no statistically significant difference was detected according to plasma glucose, estradiol level, insulin levels and HOMA-IR between the PCOS patients with different functional progesterone receptor polymorphism (PROGINS).

Coulam et al., (2008) investigated the prevalence of H770H (C/T genotype), V660L polymorphism and a 306 bp Alu insertion in exon 7 of the progesterone receptor among women with history of recurrent implantation failure to determine whether any of these polymorphisms might serve as a risk factor for implantation failure. DNA was extracted from the buccal swabs of 66 women experiencing implantation failure and 75 fertile control women. PCR amplified fragments was purified and sequenced to identify polymorphism. The frequencies for the three variants were reported to be 27% for H770H, 25% for V660L and 0% for the 306 bp Alu insertion in exon 7 among women with implantation failure compared with control women of 25% for H770H and 24% for V660L and 0% for the 306 bp Alu insertion in exon 7. No significant differences in the overall allelic or genotypic frequency of progesterone receptor variants was seen when women experiencing recurrent implantation failure were compared with control women. The H770H, V660L and PROGINS progesterone receptor polymorphisms were not concluded to be the markers that could identify women at risk for recurrent implantation.
after IVF/ET. The mutations of the progesterone receptor gene were investigated to seek further insight into their relationships with recurrent implantation failure after IVF/ET. This observation suggested that homozygous mutations were not sufficient to prevent implantation and adds further support for the lack of association of these polymorphisms and implantation failure. Moreover, among women with a H770H C/C genotype, there was an increase in the proportion of +331 G/A carriers as the number of implantation failures increased from 8.6% of women with no failed attempts to 40% among women with five or more embryo transfers with no successful pregnancies. The significant trend (p=0.03) for implantation failure with the combine progesterone receptor polymorphisms suggested a cumulative effect of the mutations.

Pearce et al., (2008) studied a total of 4788 ovarian cancer cases alongwith 7614 controls and established an international consortium to pool resources and data from many ovarian cancer case–control studies in an effort to identify variants that influence risk and examined three PGR single nucleotide polymorphisms (SNPs), for which previous data have suggested to affect ovarian cancer risk. These were þ331 C/T (rs10895068), PROGINS (rs1042838) and a 30 variant (rs608995). Unconditional logistic regression was used to calculate the association between each SNP and ovarian cancer risk. Overall, risk of ovarian cancer was not found to be associated with any of the three variants studied. However, in histopathological subtype analyses, a statistically significant association was found between risk of endometrioid ovarian cancer and the PROGINS allele (n=651, OR=1.17, 95% CI=1.01–1.36, P=0.036). A borderline evidence of an association between risk of endometrioid ovarian cancer and the +331C/T variant (n=725 cases; OR=0.80, 95% CI 0.62–1.04, P=0.100) was also observed. These data suggested that while these three variants in the PGR were not associated with ovarian cancer, the PROGINS variant might play a modest role in risk of endometrioid ovarian cancer. No statistically significant association was observed between the 30 variant (rs608995) and risk of ovarian cancer when all cases were considered. In subtype analysis, a borderline statistically significant association was observed between endometrioid cases and the rs608995 (30 variant) (OR=1.14, 95% CI 0.99–1.31, P=0.076), however, this effect was limited to individuals carrying at least one copy of the PROGINS. Across the studies, the minor allele frequencies in White controls ranged from 4.6 to 7.3% for +331C/T (rs10895068), 9.2 to 19.0% for the PROGINS (rs1042838) and 20.0 to 26.6% for the 30 variant (rs608995). In
cell type-specific subgroup analyses, a suggestive association was observed between carrying a T allele and risk of endometrioid invasive ovarian cancers (per allele OR=0.80; 95% CI 0.62–1.04; P=0.100). However, risk was statistically significantly elevated among endometrioid ovarian cancer cases (OR=1.17, 95% CI 1.01–1.36, P=0.036). In a joint effects analysis, risk of endometrioid ovarian cancer associated with the PROGINS was observed only among noncarriers of the +331 minor allele (OR=1.22, 95% CI 1.01–1.46, P=0.037). Although not statistically significant, the protective effect of the +331 minor allele persisted among non-carriers of the PROGINS (OR=0.76, 95% CI 0.55–1.06, P=0.11) and carriers of the PROGINS (OR=0.79, 95% CI 0.40–1.57, P=0.50).

Peiro et al., (2008a) carried out a candidate gene study which involved a total of 598 F2 population from a cross between the high and low lines selected divergently for uterine capacity during 10 generations of does. Uterine capacity was considered to be a main component of litter size. The progesterone receptor gene was tested as a candidate gene to determine whether or not the polymorphisms explained the differences in litter size and its components. Fragments of the promoter region and exons 1–8 were amplified and sequenced. One SNP was found in the promoter region, 2464 G>A; three SNPs in the 59-UTR exon 1 and a silent SNP in exon 7. However, the first four SNPs were segregated in two haplotypes. The 2464G>A SNP was not fixed in the H and L lines, the allele G frequency being 0.83 in the H line and 0.42 in the L line (P<0.05) and the allele G frequency being 0.75 in H parental animals and 0.29 in L parental animals. At 48 hr of gestation, the difference in EES (early embryo survival) and embryonic stage of development was small. However, at 72 hr of gestation, the GG genotype had 0.36 embryos more than the AA genotype and also had a more advanced embryonic stage of development, showing a lower percentage of compacted morulae and a higher percentage of blastocysts. The allele G found in the promoter region was found in 75% of the high-line parental animals and in 29% of the low-line parental animals. The difference in litter size between the GG and GA genotypes was similar to the difference found between homozygote genotypes; however, comparing the GG and GA genotypes at 72 hr of gestation, the GA genotype shows both EES and embryonic stage of development similar to that of the GG genotype and higher than that of the AA genotype. At implantation, the GA genotype showed a similar number of implanted embryos to that of the AA genotype and a lower number than that of the GG genotype.
Peiro et al., (2008b) evaluated the effect of the 2464 G>A SNP found in the promoter region of the progesterone receptor gene on progesterone receptor (PR) expression by Western blot analysis. This SNP was associated with two lines divergently selected for uterine capacity. The AA genotype was the genotype more frequent in the line selected to decrease uterine capacity whereas the GG genotype was the genotype more frequent in the line selected to increase uterine capacity. Two isoforms of progesterone receptor were obtained: the PR-B, previously described in rabbits, and the PR-A isoform, not described in rabbits before. The molecular weight of the PR-B was 110 kDa, similar to the isoform described by Gutierrez-Sagal et al., (1993), while the molecular weight of the PR-A was 81 kDa. The rabbit PR-A isoform has been described for the first time. A similar band was previously found by Gutierrez-Sagal et al., (1993) although they described this band as a degradation of the PR-B isoform. Higher PR expressions (both PR-A and PR-B) were obtained at day 3 of gestation in the oviduct with respect to day 2. Similar PR-A expression was observed in the ampulla and isthmus, meanwhile a higher PR-B expression was found in the isthmus. Regarding the genotypes, both PR expressions in the AA genotype were higher than in the GG genotype, being the PR-B and the PR-A expression 1.2 and 1.4 times in the AA genotype, respectively.

Pirildar et al., (2010) investigated the relationship between two different progesterone receptor gene polymorphisms, agoraphobia, nocturnal panic attacks and respiratory subtype of PD (Panic Disorder) among 98 patients diagnosed with PD (respiratory and non-respiratory subtypes) and 129 healthy controls. Seventy-six of 98 patients with PD (79.6%) were in respiratory subtype (RS) of PD. A significant association was revealed between G331A polymorphism and PD in both sexes (p=0.02; OR=2.291; CI=1.141-4.6). PROGINS Alu gene polymorphism was found to be associated with agoraphobia (p=0.002; OR= 3.8; CI=1.65-8.76). The analysis was repeated in female patients with PD. PROGINS Alu gene polymorphism was significantly associated with PD in women (p=0.036; OR=2.192; CI=1.053-4.564). The progesterone receptor gene polymorphism was suggested to be a susceptibility factor for PD. The distributions of genotypes were in the Hardy Weinberg equilibrium. G allele was more frequently found in controls than panic patients and the difference between patients and controls was significant (p=0.026; OR=2.291; CI=1.141-4.6). Furthermore, the association was investigated between PD subtypes and progesterone gene polymorphism (G331A and PROGINS Alu) comparing them with healthy controls. A nearly statistically significant
association was found between respiratory subtype and PROGINS *Alu* gene polymorphism (p=0.064; OR=1.87; CI=0.965-3.625). An association between G331A gene polymorphism and the respiratory subtype (p=0.048; OR=2.11; CI=1.008-4.453) and non respiratory subtype (p=0.046; OR=3.027; CI=1.017-9.005) of panic disorder was also detected. Also a sex related relationship between panic disorder and the frequency of T1 allele was found, which was higher in female patients with PD.

Aruna *et al.*, (2010) checked the association of the 3 linked single nucleotide polymorphisms (SNPs) of the progesterone receptor (PR) gene (exon 1: G 1031 C; S344T, exon 4: G 1978 T; L660V and exon 5: C 2310 T; H770H) and the PROGINS insertion in the intron G, between exons 7 and 8 with Recurrent Spontaneous Abortion (RSA) in the Indian population. A total of 143 women with RSA and 150 controls were sequenced for all the 8 exons looking for the above 3 linked SNPs of the PR gene earlier implicated in the RSA, as well as for any new SNPs that might be existing in the Indian population. Not any new mutation was found and also the significant role of the 2 allele (representing the mutant allele at the three SNP loci) or the T2 allele (PROGINS insertion) in the manifestation of RSA was not obtained. In addition to these an LD pattern between each of the 3 SNPs and the PROGINS insertion was also not obtained. The results further suggested that the PR gene mutations might not play any exclusive role in the manifestation of RSA and instead any significant increase was absent in the frequency of either the 2 allele or the T2 allele among RSA women of the Indian population rather found a higher frequency of the 2 allele in the controls in contrast to expectations. Furthermore, the analyses of PROGINS *Alu* insertion revealed genotypic frequencies of 133 (97.1%) homozygous wild type (T1/T1), 3 (2.2%) heterozygous (T1/T2) and 1 (0.7%) homozygous PROGINS insertion (T2/T2) among the RSA women as compared to the frequencies of 143 (95.3%), 6 (4%) and 1 (0.7%) among the controls.

Peiro *et al.*, (2010) evaluated the association of the 2464G > A SNP found in the promoter region of the rabbit progesterone receptor gene with progesterone receptor (PR) expression. This SNP was found to be associated with 2 lines divergently selected for uterine capacity; the high line selected to increase uterine capacity and the low line selected to decrease uterine capacity. Two progesterone isoforms were obtained using a commercial monoclonal antibody: the PR-B isoform (110 kDa) and the PR-A isoform (81 kDa). The GG genotype was found more frequently in the high line, showed less PR-B
and PR-A expression than the AA genotype in the oviduct (GG/AAPR-B = 0.81 and GG/AAPR-A = 0.73) and uterus (around 0.70 in both isoforms). The GA genotype showed similar PR-A expression in both tissues and also similar PR-B expression in the oviduct to that of the GG genotype. Conversely, the GG genotype showed less PR-B expression than the GA genotype in the uterus (GG/GAPR-B = 0.79). Similar expression of both PR isoforms was found in the uterus at day 2 and 3 of gestation; meanwhile, an increase of both isoforms was observed in the oviduct. Similar PR-A expression was observed in the ampulla and isthmus; meanwhile, the PR-B expression in the isthmus was doubled that in the ampulla. An interaction between the day of gestation and different parts of the oviduct was found only in the PR-B isoform. Differences between d 2 and 3 of gestation were found to be greater in the isthmus than in the ampulla. The PR-B expression at day 3 of gestation was 2.4 times the expression at day 2 of gestation and the PR-A expression was 1.6 times greater. The PR-B expression in the AA genotype was 1.2 times and the PR-A expression was 1.4 times the expression in the GG genotype.

Costa et al., (2011) investigated a possible link between endometriosis and polymorphism of the progesterone receptor gene (PROGINS). The endometriosis group consisted of 54 patients with a diagnosis of endometriosis by laparoscopy, and the control group comprised 44 women without endometriosis. When analyzing the genotype distribution of the PROGINS polymorphism group with endometriosis (N = 54) 66.7% for the A1/A1 genotype, 31.5% (17/54) for the A1/A2 genotype, 1.8% (1/54) for the A2/A2 genotype and 33.3% (18/54) of patients in the endometriosis group were found to possess polymorphic genotype for PROGINS (A1/A2+A2/A2). In the control group (N = 45), 84.5% (38/45) belonged to the A1/A1 genotype, 11.1% (5/45) had the genotype A1/A2, 4.4% (2/45) had the A2/A2 genotype and 15.5% (7/45) of these patients had the polymorphic genotype for PROGINS (A1/A2+A2/A2). The frequency of polymorphic genotypes (A1/A2 and A2/A2 combined) was significantly higher in patients with endometriosis (33%) than in the control group (16%), where this difference was statistically significant (P = 0.0351). The frequency of the polymorphic genotype A1/A2 alone of the progesterone receptor gene in the group of patients with endometriosis is about three times higher than in the control group. The frequency of genotype A2/A2 of PROGINS polymorphism is about two times higher in the endometriosis group than in the control group. When the endometriosis group was subdivided into fertile and infertile or
with regard to the degree of endometriosis, statistically significant differences were not observed.

Near et al., (2011) investigated the association between self-reported endometriosis and the putative functional promoter +331C/T SNP and the PROGINS allele among 5812 white female controls with mean ages of 55.5 years (SD 11.9 years; range 16-91 years), out of whom 348 had endometriosis. The prevalence of endometriosis was 6.0% overall (n = 348); the prevalence was between 5% and 8% in seven of the eight studies. The +331 T allele was also shown to result in a reduced progesterone (P) receptor A to receptor B ratio and suggested the importance of this ratio for this disease upon confirmation of observed association with endometriosis. The overall minor allele frequencies for +331C/T and PROGINS were also studied. Furthermore, endometriosis was found to be associated with carrying one or two copies of the +331 T allele (OR = 0.65, 95% CI = 0.43–0.98, P=0.042) whereas there was no association found between the PROGINS allele and endometriosis (odds ratio=0.94, 95% confidence interval 0.76-1.16). A 35% reduction was observed in the occurrence of endometriosis among carriers of the +331 T allele (95% CI: 2%–57%). Progesterone receptor B acted as a classic hormone receptor, mediating the effects of P, whereas PR-A acted as a repressor of PR-B and as a result, the presence of the +331 T allele was hypothesized to lead to a greater effect of P. The finding of a reduced risk was biologically plausible because endometriosis was responsive to P. The suggestive evidence was also provided for a role of variation in the PGR gene with endometriosis and indicated that P signalling might be important in this disease.

Manuck et al., (2011) hypothesized that single nucleotide polymorphisms in the human progesterone receptor (PGR) affected the response to 17-OHPC in the prevention of recurrent PTB (preterm birth). Secondary analysis of a study of 17-OHPC vs placebo for recurrent PTB prevention was also conducted. Twenty PGR gene single nucleotide polymorphisms were studied and multivariable logistic regression assessed for an interaction between PGR genotype and treatment status in modulating the risk of recurrent PTB. A total of 380 women were included; 253 (66.6%) received 17-OHPC and 127 (33.4%) received placebo. Multivariable logistic regression demonstrated significant treatment-genotype interactions (either a beneficial or harmful treatment response) for African Americans delivering <37 weeks gestation for rs471767 and rs578029 and for Hispanics/Caucasians delivering <37 weeks gestation for rs500760 and <32 weeks
gestation for rs578029, rs503362, and rs666553. Furthermore, the clinical efficacy and safety of 17-OHPC for recurrent PTB prevention might be altered by PGR gene polymorphisms.

Lee et al., (2012) investigated the roles of posttreatment serum progestogen increase and progesterone receptor gene (PGR) genetic variations on changes in mammographic density after adjusting for serum estrone and estrone sulfate levels in 280 Postmenopausal Estrogen/Progestin Interventions trial participants randomized to EPT (Estrogen plus progestin therapy) treatment. The increase in posttreatment serum progestogen level was found to be associated positively with greater increases in mammographic density after adjustment for covariates (P trend = 0.044). Compared with women in the lowest quartile of serum progestogen level, women in the highest quartile experienced a 3.5% greater increase in mammographic density (P = 0.046). A strong indication was not found for the genetic variation in PGR regarding its association with mammographic density increase or modifying the association with serum progestrogen.

Hein et al., (2012) assessed effect modification of MHT (Menopausal Hormone Therapy) use by five coding single nucleotide polymorphisms (SNPs) in the progesterone metabolizing enzymes AKR1C3 (rs7741), AKR1C4 (rs3829125, rs17134592), and SRD5A1 (rs248793, rs3736316) using a two-center population-based case–control study from Germany with 2,502 postmenopausal breast cancer patients and 4,833 matched controls. Breast cancer risk associated with duration of combined therapy was significantly modified by SRD5A1_rs3736316, showing a reduced risk elevation in carriers of the minor allele (P interaction, empirical-Bayes = 0.006 using the empirical-Bayes method, P interaction, logistic regression = 0.013 using logistic regression). The risk associated with duration of use of monotherapy was increased by AKR1C3_rs7741 in minor allele carriers (P interaction, empirical-Bayes = 0.083, p interaction, logistic regression = 0.029) and decreased in minor allele carriers of two SNPs in AKR1C4 (rs3829125: P interaction, empirical-Bayes = 0.07, P interaction, logistic regression = 0.021; rs17134592: P interaction, empirical-Bayes = 0.101, P interaction, logistic regression = 0.038). After Bonferroni correction for multiple testing only SRD5A1_rs3736316 assessed using the empirical-Bayes method remained significant. Postmenopausal breast cancer risk associated with combined therapy would be further modified by genetic variation in SRD5A1.
Saint-Dizier et al., (2012) characterised the expression of PR (progesterone receptor), PGRMC1 (progesterone receptor for membrane component) and PGRMC2 in the bovine oviduct during the post-ovulation period, and related their expression to the presence of an embryo, the proximity of the CL and to the region of the oviduct via two experiments. In the first experiment, whole oviduct sections were collected from Holstein cows at Day 1.5, Day 4 and Day 5 post-ovulation (n = 2 cows per stage). The expression of PR, PGRMC1 and PGRMC2 was studied in the ampulla and isthmus by RT-PCR, western-blot and immuno-histochemistry. The PR, PGRMC1 and PGRMC2 were found to get expressed in all oviduct samples. PGRMC1 was mainly found localised in the luminal epithelium whereas PR and PGRMC2 were localised mainly in the epithelium along with the muscle and stroma layers of the oviduct. The expression was primarily observed to be nuclear for PR, cytoplasmic for PGRMC1 and both nuclear and cytoplasmic for PGRMC2. In second experiment, oviduct epithelial cells were collected from cyclic and pregnant Charolais cows (n = 4 cows per status) at Day 3.5 post-ovulation and mRNA expression of PR, PGRMC1 and PGRMC2 was examined in the ampulla and isthmus by real-time quantitative PCR. The mRNA levels for PR, PGRMC1 and PGRMC2 were checked and not found to be affected by either the pregnancy status or by the side relative to the CL. However, the expression of PR and PGRMC2 varied with the region of the oviduct: PR was expressed in the isthmus at a higher level whereas PGRMC2 was highly expressed in the ampulla. It was further suggested that P4 (progesterone) regulated the functions of the bovine oviduct in a region-specific manner and through both classical and non classical pathways during the post-ovulation period.

Seleem et al., (2014) determined the frequency of the PROGINS polymorphism and the estrogen receptor b gene (ERP) +1730 G/A polymorphism in infertile women with endometriosis and fertile women. The study included 50 women with endometriosis and 24 fertile women (control). PCR-RFLP was used to identify the PROGINS polymorphism and the ERP gene + 1730 G/A polymorphism. Genotypes A1A1, A1A2 and A2A2 (A2 representing the PROGINS polymorphism) of the progesterone receptor gene presented frequencies of 88%, 10% and 2%, respectively, in women with endometriosis. The allelic frequency of the PROGINS polymorphism was found lower in women with endometriosis (P > 0.05) as compared to healthy females in the patients with minimal/mild endometriosis (p = 0.978), 84.4 %, 12.5 % and 3.1 %, respectively, among the patients with moderate/severe endometriosis (p = 0.595); 94.4%, 5.6% and 0.0%, respectively, and
83.3%, 12.5 % and 4.2%, respectively, in the control group. Genotypes GG, GA and AA of the ERP gene presented frequencies of 56%, 34% and 10%, respectively, in the women with endometriosis (p < 0.05). In the control group; 91.8 % presented the normal homozygous genotype GG, 8.2% the GA heterozygous genotype and 0.0 % the homozygous mutated genotype AA. It was suggested that the estrogen receptor gene (ERP) +1730 G/A polymorphism might be associated with risk of endometriosis.

Pabalan et al., (2014) evaluated the association between the PROGINS polymorphism and the risk of endometriosis. A meta-analysis of 12 published case-control studies with a total sample size of 3,321 (1,323 cases/1,998 controls) was performed. The risk (odds ratio [OR] 95 % confidence intervals) of endometriosis association with the PROGINS polymorphism was estimated. An association was found between the presence of the variant allele and risk of endometriosis which was more in the homozygous and recessive models (OR 1.41-1.43, p = 0.15-0.17), and less in the dominant and co-dominant models (OR 1.22, p = 0.11-0.15). Reanalysis without the studies whose controls deviated from the Hardy-Weinberg Equilibrium did not found to alter the dominant and co-dominant effects (OR 1.19-1.22, p = 0.19-0.32), but exacerbated the homozygous and recessive effects (OR 1.59, p = 0.09). The subgroups based on geography showed increased risk associations, which was consistently significant in the European (OR 1.52-2.72, p = 0.0008-0.03) but not in the Brazilian studies, where ORs ranged from reduced (OR 0.70-0.74, p = 0.54-0.61) to increased (OR 1.11, p = 0.75) risks. Heterogeneity was confined in all comparisons to the dominant and co-dominant models (I (2) = 38-70 %), except in the European subgroup, having zero heterogeneity (I (2) = 0 %) in all genetic models, as did all homozygous and recessive effects.