(54) Title: RECOMBINANT ANTI-LHRH VACCINES

(57) Abstract: The present invention relates to oligonucleotide sequences of SEQ ID Nos. 1 and 2, and polypeptide sequences of SEQ ID No. 3 and 4, useful for preparing recombinant anti-LHRH DNA and proteinic vaccines; also, a method of obtaining the vaccines using oligonucleotide sequence of SEQ ID No. 1 or 2; further, the vaccine compositions comprising DNA and/or proteinic vaccines optionally along with an adjuvant and/or delivery system; and lastly, a method of reducing levels of sex steroid hormones by eliciting an effective antibody response against LHRH in mammals of both sexes using the vaccine compositions, for controlling and/or treating the sex-steroid hormone dependent benign prostatic hypertrophy and/or prostate cancer, breast cancer, estrus of companion animals, or meat quality of male animals.
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RECOMBINANT ANTI-LHRH VACCINES

Field of the present invention

The present invention relates to oligonucleotide sequences of SEQ ID Nos. 1 and 2, and polypeptide sequences of SEQ ID No. 3 and 4, useful for preparing recombinant anti-LHRH DNA and proteinic vaccines; also, a method of obtaining the vaccines; further, a vaccine compositions comprising DNA and/or proteinic vaccines optionally along with an adjuvant and/or delivery system; and lastly, a method of reducing levels of sex steroid hormones useful for controlling and/or treating the sex-steroid hormone dependent benign prostate hypertrophy and/or prostate cancer, breast cancer, estrus of companion animals and meat quality of male animals.

Background and prior art references of the present invention

As the life span of the people lengthens in India and around the world, the number of males experiencing prostate hypertrophy increases. Prostate is an unusual organ, which enlarges with age in contrast to most of the functions of the body that regress. Every male who lives long enough, will experience prostate-hypertrophy and a percentage amongst them will develop carcinoma of prostate. In United States of America, prostate cancer has already become the largest killer of men due to cancer. In view of these physiological sum pathological anomalies associated with ageing males, worldwide, there is a need for effective and more convenient treatment regimens to cope with this problem.
At present, prostate hypertrophy and cancers are treated by surgery and drugs, which entail hospitalization costs. A number of anti-androgens are also used. Luteinizing hormone releasing hormone (LHRH) analogues, are found to be of great therapeutic value, they are however costly. As the prostate-hypertrophy and hyperplasia inflicts both rich and poor invariably in advancing years, low cost treatments would have special interest, particularly in economically developing countries like India.
Injecting a vaccine to generate antibodies reactive with mammalian hormone LHRH or GnRH, has potential for the immuno-therapy of prostate cancer as well as for atrophy of the
Injecting a vaccine to generate antibodies reactive with mammalian hormone LHRH or GnRH, has potential for the immuno-therapy of prostate cancer as well as for atrophy of the benign prostate enlargement. The hormone (LHRH/GnRH) is largely produced by hypothalamus in the brain, from where it travels through blood circulation to pituitary, an endocrine gland situated at the base of skull. Under the influence of LHRH/GnRH, the pituitary produces and secretes two gonadotropins – FSH and LH, which in turn act on the testes in males, and ovaries in females to generate sperm or eggs, respectively.

Concomitantly the hormonal cascade also causes the production of sex steroid hormones: testosterone in males, and estrogen and progesterone in the females. These sex steroids provide an important stimulus to the accessory reproductive organs. In males, androgens promote the growth and sustain the size of the prostate, besides other accessory organs. Blocking of LHRH results in blocking of production of sperms and testosterone acting on accessory reproductive organs, such as prostate. In females, blocking of LHRH blocks ovulation and production of female sex hormones such as estrogens which promote the growth of hormone dependent breast cancers.

Besides this principle pathway of hypothalamus-pituitary-gonad interaction, there is growing evidence to indicate that LHRH is also produced in small amounts by other tissues such as testes and placenta, and that LHRH exercises an additional direct effect on the prostate (Fuerst et al., 1997; Talwar et al., 1999). Therefore, by generation of antibodies competent to bind with LHRH and neutralize its action, the growth promoting effect of androgens would be negated, over and above, the cancellation of the direct action of LHRH on this organ. In the females, inactivation of LHRH by bioeffective antibodies, would block ovulation and production of female sex steroids. The vaccine engendering such antibodies would have potential therapeutic applications in hormone-dependent breast cancers and in endometriosis where therapy with LHRH antagonists has been found useful.

The companion animals like dogs and cats, also secrete LHRH. Blocking LHRH by the bioeffective antibodies, would provide reversible control of fertility and hormone dependent manifestations such as estrus of female companion animals (Talwar et al., 1985). Immunization of male animals against LHRH will also have application in blocking the
testosterone production thereby in improving the quality of meat in animals raised for this purpose like pigs, rams, etc.

LHRH or GnRH is common to both males and females and interestingly its molecular structure is conserved and nearly identical in animals and humans. Thus rat offers a model for evaluation of the efficacy and safety of any product designed against LHRH for eventual application in humans. This axiom has been proven by our prior work on a semi-synthetic vaccine against LHRH (Talwar et al., 1992), which caused atrophy of the prostate in rats (Jayashankar et al., 1989) and monkeys (Giri et al., 1991). It inhibited the growth of the Dunning prostatic tumors implanted in rats (Fuerst et al., 1997). This very vaccine in clinical trials was found safe and effective in patients suffering from advanced stage carcinoma of prostate as per the trials conducted at the Postgraduate Institute of Medical Research, Chandigarh and the All India Institute of Medical Sciences, New Delhi, India as well as in Salzburg General Hospital, Austria (Talwar et al., 1999).

It was discovered that a semi-synthetic anti-LHRH vaccine designed for the purpose, caused atrophy of the prostate (Talwar et al., 1992). One disadvantage associated with this semi-synthetic vaccine, was that it was costly to make. Also, it required a chemical linkage with a carrier like diptheria toxoid (DT) or tetanus toxoid (TT). This resulted in further loss of yield coupled with inconsistencies of site of linkage. The use of DT/TT suffered from an additional disadvantage of engendering carrier-induced suppression of immune response to LHRH on repeated immunization (Gaur et al., 1990; Sad et al., 1991).

The present vaccine, which is hereby communicated, is designed by recombinant DNA technology to enable large-scale production at low costs. It would be free of inconsistencies of carrier linkage, and avoid carrier induced epitopic suppression.

**Objects of the present invention**

The main object of the present invention is to design oligonucleotide sequences useful in preparing recombinant anti-LHRH DNA vaccines.

Another main object of the present invention is to design polypeptide sequences useful in preparing recombinant anti-LHRH proteinic vaccines.
Yet another object of the present invention is to develop a method of obtaining purified recombinant anti-LHRH DNA vaccine.

Still another object of the present invention is to develop a method of obtaining purified recombinant anti-LHRH proteinic vaccine.

Still another object of the present invention is to develop a vaccine composition using purified recombinant anti-LHRH DNA optionally along with an adjuvant and/or a delivery system.

Still another object of the present invention is to develop a vaccine composition using purified recombinant anti-LHRH protein optionally along with an adjuvant and/or a delivery system.

Still another object of the present invention is to develop a vaccine to decrease the levels of sex-steroid hormones.

Still another object of the present invention is to develop a vaccine to decrease the levels of sex-steroid hormone testosterone.

Still another object of the present invention is to treat sex-steroid hormone dependent cancers.

Still another object of the present invention is to bring about atrophy of the enlarged prostate.

Still another object of the present invention is to improve the quality of the meat in male animals.

Still another object of the present invention is to treat sex hormone dependent breast cancer.

Still another object of the present invention is to regulate estrus of companion animals, such as dogs.

**Summary of the present invention**

The present invention relates to oligonucleotide sequences of SEQ ID Nos. 1 and 2, and polypeptide sequences of SEQ ID No. 3 and 4, useful for preparing recombinant anti-LHRH DNA and proteinic vaccines; also, a method of obtaining the vaccines using oligonucleotide sequence of SEQ ID No. 1 or 2; further, the vaccine compositions comprising DNA and/or proteinic vaccines optionally along with an adjuvant and/or delivery system; and lastly, a method of reducing levels of sex steroid hormones by eliciting an effective antibody response against LHRH in mammals of both sexes using the vaccine compositions, for controlling and/or treating the sex-steroid hormone dependent benign prostate hypertrophy and/or prostate cancer, breast cancer, estrus of companion animals, and meat quality of male animals.
Detailed description of the present invention

Accordingly, the present invention relates to oligonucleotide sequences of SEQ ID Nos. 1 and 2, and polypeptide sequences of SEQ ID No. 3 and 4, useful for preparing recombinant anti-LHRH DNA and proteincic vaccines; also, a method of obtaining the vaccines using oligonucleotide sequence of SEQ ID No. 1 or 2; further, the vaccine compositions comprising DNA and/or proteincic vaccines optionally along with an adjuvant and/or delivery system; and lastly, a method of reducing levels of sex steroid hormones by eliciting an effective antibody response against LHRH in mammals of both sexes using the vaccine compositions, for controlling and/ or treating the sex-steroid hormone dependent benign prostate hypertrophy and/or prostate cancer, breast cancer, estrus of companion animals, and meat quality of male animals.

SEQ ID No. 1
ATGGACATGGAGCTATGGCCTGCGTCCGGGCGGTAGCGGTGATATCAGAAAAA
AAAATCGCGAAAAATGAAAAAGCAGACGGCGGTGTTTAACTGGTGAACCGTGAAG
CTTAGGAGGAACATTCGAGCTATGGCCCTGCGTCCGGGCGGCGGTGAGCATCGGAAATAT
AACGTGTTTCATAAACAAAACCTTTGAAACTGCGCCGCGGCGGTGGAACATCGGA
GCTATGGCCCTGCGCCGGGCGGTGAGCTATATCAAAAGCGAAACAGCAAAATTAT
CGGCATTACCAGAATCGGAGCTACGCGGTGAACATGTCATGCGTCACGTGCTGCGGCG
GTTAGCGGTCTCGAGCGGAATCAAAGGCCGTAGATCGTCAGTCGTCGGAAGCGTG

SEQ ID No. 2
GAATTCATGGAGATATCGGAAAAAAATCGCGAAAAATGAAAAAGCAGACGGCGGTG
TTTACGTTGGAACGCGTGGTGAGATCAATGCTGAGCTATGGGCTGCTGCCGCGGTG
GTGATCAAGGTGCTTTCAGCGGCTTCACCAGCAGCTGTGGAAGCTTAGCGATGC
GCTGATCGGTGGTGAACATTCGAGCTATGCGCTGCCGCGGGGTGTCGAGTATATC
AAAGCGAACAGCAAAATTATGCGCATCAACGGAAACTGGGTTGGAACATCGGAAGC
TATGCTGCTGCGCCGGGTTGGAAGCGAACATAATCGGTTTCTCATACAAACCGCTTTG
AATGCGCGCGTGGGTTGGAACATTCGAGCTATGGCTGCGTCCGCGGGGTGCGGCT
GAGCGAAATCAAAGGCCGTGATCGTCAGTCGTCGAGAAGGGTGTGCGGTGAAACA
TTGGAGCTATGCGCTGCGTCCGCGG
SEQ ID NO. 3
MEHW SYGLRPGGSGDIEKKI AKMEKASSVFNVNVNGKLSGEHWSYGLRPGGQQYIKANSKFIGITELGSGEHWWSYGLRPGGSGLSEIKGVIVHRLEGVGS

SEQ ID No. 4
EFMDIEKKIAKMEKASSVFNVNVNSGGEHWWSYGLRPGGDQVHFPQLPPAVVKLSDALIGGEHWWSYGLRPGGQYIKANSKFIGITELGGEHW

In an embodiment of the present invention, wherein an oligonucleotide sequence of SEQ ID No. 1 useful for preparing a recombinant anti-LHRH DNA vaccine.

In another embodiment of the present invention, wherein an oligonucleotide sequence as claimed in claim 1, wherein the said SEQ ID No. 1 expressing corresponding polypeptide sequence of SEQ ID No. 3 useful as a recombinant anti-LHRH proteinic vaccine.

In yet another embodiment of the present invention, wherein a polypeptide sequence of SEQ ID No. 3 useful for preparing a recombinant anti-LHRH proteinic vaccine.

In still another embodiment of the present invention, wherein an oligonucleotide sequence of SEQ ID No. 2 useful for preparing a recombinant anti-LHRH DNA vaccine.

In still another embodiment of the present invention, wherein an oligonucleotide sequence as claimed in claim 4, wherein the said SEQ ID No. 2 expressing corresponding polypeptide sequence of SEQ ID No. 4 useful as a recombinant anti-LHRH proteinic vaccine.

In still another embodiment of the present invention, wherein a polypeptide sequence of SEQ ID No. 4 useful for preparing a recombinant anti-LHRH proteinic vaccine.

In still another embodiment of the present invention, wherein a method of obtaining purified recombinant anti-LHRH DNA vaccine using oligonucleotide sequence of SEQ ID No. 1 or 2, said method comprising the steps of:

- designing an oligonucleotide sequence of SEQ ID No. 1 or 2,
- inserting the said sequence into a eukaryotic expression vector comprising an appropriate promoter,
- replicating the expression vector containing the oligonucleotide sequence of SEQ ID No.1 or 2 in a endonuclease-free bacterial host,
- extracting plasmid DNA from the bacterial host of step (e), and
- purifying a plasmid DNA to obtain the DNA vaccine.

In still another embodiment of the present invention, wherein the promoter is selected from a group comprising SV40, CMV, HSV, RSV, and MMTV.

In still another embodiment of the present invention, wherein the bacterial host is selected from a group comprising E. coli DH1, DH5alpha, C600, and XL1-Blue.

In still another embodiment of the present invention, wherein the vectors is selected from a group comprising VR1012, VR1020, pRc/CMV, pUMVC7 and pVAC.

In still another embodiment of the present invention, wherein a method of obtaining recombinant anti-LHRH proteinic vaccine using polypeptide of SEQ ID No. 3 or 4, said method comprising the steps of:

- designing an oligonucleotide sequence of SEQ ID No. 1 or 2,
- inserting the said sequence into a prokaryotic expression vector comprising an appropriate promoter,
- replicating the expression vector containing the oligonucleotide sequence of SEQ ID No.1 or 2 in a protease-free bacterial host,
- extracting recombinant protein from the bacterial host of step (e), and
- purifying and refolding the recombinant proteins to obtain the proteinic vaccine having polypeptide sequence of SEQ ID No. 3 or 4.

In still another embodiment of the present invention, wherein the promoter is selected from a group comprising T7, T5, tac, lac, pH, and \( \lambda P_L \).

In still another embodiment of the present invention, wherein the bacterial host is selected from a group comprising E. coli strains of BL21, BL21(DE3), BL21(DE3)pLys, TG1, and XL1-Blue.

In still another embodiment of the present invention, wherein the vectors are selected from a group comprising pRSET, pET, pQE, and pSE420.
In still another embodiment of the present invention, wherein a vaccine composition comprising plasmid DNA containing oligonucleotide sequence of SEQ ID No. 1 optionally along with an adjuvant and/or delivery system useful as a recombinant anti-LHRH DNA vaccine.

In still another embodiment of the present invention, wherein a vaccine composition comprising protein of polypeptide sequence of SEQ ID No. 3 optionally along with an adjuvant and/or delivery system useful as a recombinant anti-LHRH proteinic vaccine.

In still another embodiment of the present invention, wherein a vaccine composition comprising plasmid DNA containing oligonucleotide sequence of SEQ ID No. 2 optionally along with an adjuvant and/or delivery system useful as a useful as a recombinant anti-LHRH DNA vaccine.

In still another embodiment of the present invention, wherein a vaccine composition comprising protein of polypeptide sequence of SEQ ID No. 4 optionally along with an adjuvant and/or delivery system useful as a recombinant anti-LHRH proteinic vaccine.

In still another embodiment of the present invention, wherein a safe, economical, and effective method of reducing levels of sex steroid hormones by eliciting an effective antibody response against Luteinizing hormone Releasing Hormone (LHRH) in mammals using either DNA vaccine containing oligonucleotide sequence of SEQ ID No. 1 or 2, or proteinic vaccine having polypeptide sequence of SEQ ID No. 3 or 4, said method comprising steps of administering said DNA or proteinic vaccine optionally along with an adjuvant and/or a delivery system to the mammal through parenteral route, and thereby reducing levels of sex steroid hormones.

In still another embodiment of the present invention, wherein the delivery system is selected from a group comprising Microparticles of poly lactide, poly glycolide or poly-lactic-co-glycolic acid (PLGA) copolymer, Nano particles of calcium phosphate, Cationic transfection lipids, and Gene gun.

In still another embodiment of the present invention, wherein the sex hormones to be reduced are, testosterone in males, and progesterone and estrogen in females.

In still another embodiment of the present invention, wherein the said method is used for controlling and/or treating the sex-steroid hormone dependent cancers.
In still another embodiment of the present invention, wherein said method brings down the levels of testosterone to castration levels without surgical removal of testes.

In still another embodiment of the present invention, wherein said method leads to atrophy of the enlarged prostate.

In still another embodiment of the present invention, wherein the said method is employed in the treatment of prostrate cancer.

In still another embodiment of the present invention, wherein said method is employed to suppress heat/estrus in female companion animals.

In still another embodiment of the present invention, wherein said method is employed to improve meat quality of animals.

In still another embodiment of the present invention, wherein the said method is employed to treat hormone dependent breast cancer.

In still another embodiment of the present invention, wherein the said method is used for reversible control of fertility in male animals.

In still another embodiment of the present invention, wherein parenteral route comprises intramuscular, intradermal, subcutaneous, intranasal, and oral route.

In still another embodiment of the present invention, wherein multiple injections of the vaccine can be given over an extended periods of time.

In still another embodiment of the present invention, wherein dosage of both DNA and proteinic vaccine is ranging between 20 to 500 µg per dose once or twice in a month.

In still another embodiment of the present invention, wherein the adjuvants are selected from a group comprising alum, sodium phthalyl lipopolysaccharide (SPLPS), Freund’s complete adjuvant/Incomplete Freund’s adjuvant and other commercially available adjuvants.

The present invention relates to a new vaccine, in which the DT or TT, as carriers, have been replaced by a set of peptides which can bring in the T helper cells without the risk of causing immuno-suppression of antibody production. Furthermore, the choice of these peptides has been made in a manner so as to evoke immune response by individuals of different genetic background. This innovation will enlarge the positivity of antibody response of the new vaccine and avoid the unwelcome suppression, which the use of the entire DT and TT toxoids as carriers bring in.
Breast cancer

LHRH or GnRH is common to both males and females. This is of advantage as the anti-LHRH vaccines designed for use in males will also have application in females. According to prior art, LHRH agonists alone or in combination, have been used to block estrogen and progesterone thereby controlling the breast cancer in women (Spicer and Pike, 2000; Burger et al., 1996). With LHRH agonists combined with tamoxifen, Klijn et al. (2001) achieved immunocastration equivalent to surgical castration, in women with advanced breast cancer. This endocrine therapy yielded significant progression-free survival benefit (6.8 years) in clinical trials.

Since our previously developed anti-LHRH semi-synthetic vaccine (Talwar et al., 1992) was able to react and inhibit gonadotropins in women (Gual et al., 1997) thus, it is understandable without any doubt that recombinant anti-LHRH vaccines will be useful in the treatment of hormone dependent breast cancer. In the instant Application, the Applicants have been able to establish that both DNA and proteinic vaccines are able to bring down the levels of LHRH.

Thus, it can be easily concluded that the vaccine will be able to treat the breast cancer in women.

 Estrus

LHRH is a conserved molecule; the sequence of the decapeptide is similar in humans and animals. Therefore, any LHRH based regimen designed for humans, will also have potential in affecting the LHRH controlled biological phenomena in animals. According to a prior art (Talwar et al., 1985), a monoclonal antibody generated against LHRH/GnRH, was used to suppress heat/estrus of female dogs. A single intravenous injection of ascites fluid containing anti-LHRH antibody, caused decrease in the receptivity of the female to the male and in the male’s attraction to the female, within 48 h. All the females had low levels of progesterone or estradiol in plasma and did not proceed to estrus as compared to dogs, which were not injected with the anti-LHRH antibody. Therefore, recombinant anti-LHRH vaccines generating bioeffective anti-LHRH antibodies can be used to control heat/estrus of female companion animals thereby restricting their overpopulation.

It is well established in the art that the vaccine against LHRH is effective to generate anti-LHRH antibody. Thus, it can be easily and unarguably assumed that the recombinant anti-
LHRH vaccines prepared by using oligonucleotide sequence of SEQ ID No. 1 and 2, and polypeptide sequence of SEQ ID No. 3 and 4, are capable of suppressing estrus/heat in female companion animals.

Reversible control of fertility

Recombinant anti-LHRH vaccines can also be used in providing reversible control of male fertility in addition to the management of prostate carcinoma. According to a prior art (Rovan et al., 1992), LHRH peptide was coupled to diphtheria toxoid (DT) and used to immunize rats at 4-week intervals. This method caused significant reduction in weights of reproductive organs without any side-effects. The histopathology revealed marked changes in the gonads and the accessory sex organs including the prostate. Spermatogenesis and fertility was restored after 300 days of vaccination along with below normal prostate recovery when the antibodies were no longer in circulation.

Thus, the anti-LHRH vaccines prepared by using oligonucleotide sequence of SEQ ID No. 1 and 2, and polypeptide sequence of SEQ ID No. 3 and 4, can be employed if required for reversible control of male fertility.

Meat quality

In farm animals also, testosterone associated traits have been suppressed in order to improve the meat quality. According to a prior art, Oonk et al. (1998) developed a LHRH/GnRH like peptide which they injected into the male pigs. It neutralized GnRH thereby reducing the testosterone concentration thus preventing the androgen-induced odour. Therefore, anti-LHRH vaccines prepared by using oligonucleotide sequence of SEQ ID No. 1 and 2, and polypeptide sequence of SEQ ID No. 3 and 4, which cause reduction in testosterone levels, and thus, will also have application in meat industry, for improving the quality of the meat.

Accordingly, the present invention provides an embodiment of DNA fragments comprising SEQ. ID. No. 1 or SEQ. ID. No. 2 useful for the preparation of proteinic and DNA vaccines for the treatment of prostate cancer dependent on LHRH/GnRH and testosterone in mammals like humans.

Further an embodiment of the invention features methods capable of providing a process for immunological castration without surgery.
An embodiment of the invention provides methods capable of providing a process for reducing prostate size without surgery.

An embodiment of the invention features methods capable of providing products and processes for the treatment of prostate cancer, dependent on sex-steroid hormone, testosterone, in men.

An embodiment of the invention features methods capable of providing products and processes for the treatment of breast cancer, dependent on sex-steroid hormones, estrogen and progesterone, in women.

An embodiment of the invention features methods also capable of providing products and processes for the reversible control of fertility and hormone dependent manifestations such as estrus/heat of female companion animals like dogs and cats.

An embodiment of the invention features methods capable of providing products and processes for blocking the testosterone production to improve the quality of meat in animals raised for this purpose like pigs, rams, etc.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly used by one of ordinary skill in the art to which this invention belongs. Although suitable methods and materials for the practice and testing of the present invention are described below, other methods and materials similar or equivalent to those described herein, which are well known in the art, can also be used. All publications, patents, and other references mentioned herein are incorporated by reference in their entirely. In case of conflict, the present specification, including definitions will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting. The following terms are intended to be defined as indicated below.

In still another embodiment of the present invention, wherein prostate cancer or carcinoma of prostate refers to a disease pertaining to males in which prostate cells multiply at unusual rate resulting in enlargement of prostate gland or tumor formation.

In still another embodiment of the present invention, wherein “DNA vaccine” is a composition, i.e., plasmid DNA which is free of the genes that are present in the genome of
the bacterial host used to amplify, extract and purify the plasmid DNA. The term therefore includes a recombinant DNA incorporated into an autonomously replicating plasmid/vector. It also includes a separate molecule such as cDNA, a genomic fragment, a fragment produced by polymerase chain reaction, or a restriction fragment. The isolated DNA is substantially free of other cellular components of the bacterial host (e.g., free from protein components), or the culture medium when used to grow the bacteria.

In still another embodiment of the present invention, wherein DNA vaccination means a method in which DNA containing the sequences encoding for an “antigenic determinant”, is administered to the recipient. Such methods can result in the expression of the antigenic determinant in the recipients thereby eliciting an immune response.

In still another embodiment of the present invention, wherein “Proteinic vaccine” refers to a composition, i.e., protein, which is free from other proteins that are present in the bacterial host used to extract and purify the protein. The term therefore includes a recombinant protein incorporated into a bacterial host through an autonomously replicating plasmid/vector. The isolated protein is substantially free of other cellular components of the bacterial host (e.g., free from DNA, RNA components), or the culture medium when used to grow the bacteria.

In still another embodiment of the present invention, wherein protein vaccination means a method in which recombinant protein containing an “antigenic determinant”, is administered to the recipient. Such methods can result in the expression of anti-antigenic determinant in the recipients thereby eliciting an immune response.

In still another embodiment of the present invention, wherein an “antigenic determinant “ refers to any agent, which can elicit an immunological response in an individual. The immunological response may be mediated by B- and/or T-lymphocytic cells. As used herein, the “antigenic determinants” are generally used to refer to a part of protein molecule, which contains LHRH peptides linked with other T cell peptides. T-cell peptides are those features which induce a T-cell response.

In still another embodiment of the present invention, wherein an “antibody” is used to imply immunological response, which is generated when an antigenic determinant is administered to a recipient. The antibody is generally specific to the “antigenic determinant” in terms of reactivity.
In still another embodiment of the present invention, wherein an “endonuclease” refers to an enzyme found in bacteria which causes nicks in the DNA thus making it redundant for its biological utility.

In still another embodiment of the present invention, wherein a “protease” refers to an enzyme found in bacteria which degrades proteins thus making it redundant for its biological utility.

In still another embodiment of the present invention, wherein a “nucleotide sequence” refers to single- or double-stranded DNA and RNA sequences. The term describes molecules that include any of the known bases of DNA and RNA. The nucleotide sequence is transcribed (in the case of DNA) and translated (in the case of mRNA) into peptide or protein when ‘operably linked’ to appropriate regulatory sequences.

In still another embodiment of the present invention, wherein Promoter, Intron and Polyadenylation sequences, ribosome binding site, along with other regulatory sequences, are required for the transcription and translation of the nucleotide sequence in the recipient cell.

In still another embodiment of the present invention, wherein a “eukaryotic expression vector” is referred to circular DNA molecules, i.e. plasmids, which can replicate autonomously inside an appropriate eukaryotic host. This can be used to carry desired nucleotide sequence for its expression in the recipients. Promoter, intron, termination and polyadenylation sequences, can also be present in the vector.

In still another embodiment of the present invention, wherein “Operably linked” refers to an arrangement of elements wherein the components so described are configured so as to perform their usual function. Thus, the control sequences operably linked to a coding sequences, are capable of affecting the expression of the nucleotide sequence.

In still another embodiment of the present invention, wherein an “adjuvant” is a compound or mixture of compounds, which enhances the ability of a vaccine to elicit an immune response.

In still another embodiment of the present invention, wherein “delivery system” refers to mode of vaccine delivery.

In still another embodiment of the present invention, wherein before describing the present invention in detail, it is to be understood that the terminology used herein is for the purpose of describing particular features of the invention only, and is not intended to be limiting.
In still another embodiment of the present invention, wherein nucleotide sequence of SEQ. ID. No. 1, which is useful for the preparation of DNA and proteinic vaccines competent to generate anti-LHRH response after insertion into a eukaryotic vector and prokaryotic vector, respectively, and injection as per the procedures described in the text.

In still another embodiment of the present invention, wherein nucleotide sequence of SEQ. ID. No. 2, which is useful for the preparation of DNA and proteinic vaccines competent to generate anti-LHRH response after insertion into a eukaryotic vector and prokaryotic vector, respectively, and injection as per the procedures described in the text.

In still another embodiment of the present invention, wherein the present invention provides a method for eliciting an immune response against a selected antigenic determinant using DNA and protein vaccine techniques, in rats, which are studied as animal models. As humans and rats have the same antigenic determinant, the method proven in rats, can be extended to humans to provide a suitable therapeutic immune response in the treatment of prostate or breast cancers, which are sex steroid hormone-dependent tumors.

**Brief description of the accompanying drawings**

The invention is being further elaborated with the accompanying drawings

**Figure 1** shows the schematic construct of a typical though not unique recombinant plasmid, useful for the preparation of DNA vaccine. The plasmid contains a DNA fragment corresponding to nucleotide sequence of SEQ. ID. No. 1 or SEQ. ID. No. 2.

**Figure 2** shows the schematic construct of a typical though not unique recombinant plasmid, useful for the preparation of proteinic vaccine. The plasmid contains a DNA fragment corresponding to nucleotide sequence of SEQ. ID. No. 1 or SEQ. ID. No. 2.

**Figure 3** shows generation of antibodies against LHRH in rats injected with the DNA vaccine (A) containing DNA fragment of SEQ. ID. No.1 (B) containing DNA fragment of SEQ. ID. No.2. Note that each of the eight animals has produced antibodies reactive with LHRH, and the antibody response is of long duration after two intramuscular injections of the DNA vaccine.
Figure 4 shows the decline of testosterone levels in 4 different animals immunized with the plasmid DNA vaccine. Note that testosterone declines drastically in each animal after immunization.

Figure 5 shows generation of antibodies against LHRH in rats injected with the proteinic vaccine (A) containing DNA fragment of SEQ. ID. No.2 (B) containing DNA fragment of SEQ. ID. No.1. Note that each of the eight animals has produced antibodies reactive with LHRH.

Figure 6 shows the decline of testosterone levels in 4 different animals immunized with the proteinic vaccine. Note that testosterone declines drastically in each animal with the emergence of antibodies.

Figure 7 shows the histology of a representative extirpated prostate lobe in comparison to that of a normal prostate isolated from a rat, which was not immunized. It can be seen from the diagram that immunization causes shrinkage in prostate tissue mass.

Designing the nucleotide sequence of the DNA fragment to encode for an antigenic determinant

The nucleotide sequence of the DNA fragment selected in the present invention to encode for an antigenic determinant, was designed in such a way that 5 repeat units of nucleotide sequence encoding LHRH peptide, were interspersed among the nucleotide sequences encoding 5 different small peptides recognizing determinants on the T type of lymphocytes, which play a crucial helper role in motivating the antibody generator B cells to produce antibodies.

Two designs of the DNA fragment having sequence of SEQ. ID. No. 1 and SEQ. ID. No. 2, encoding for antigenic determinant (i.e. multimeric LHRH peptides) used in the present invention. The nucleotide sequence corresponding to 2 designs differed in the placement of T-cell peptides amongst the repeat units of LHRH peptide.

Two DNA fragments corresponding to the designed nucleotide sequences, were synthesized by using the known techniques of genetic engineering in the art, like joining short fragments resulting from custom synthesized over-hanging nucleotides followed by filling in by
polymerase chain reaction technique. The DNA fragments have unique EcoR I and Sal I restriction enzyme sites in the beginning and end, respectively, to facilitate insertion of these fragments into an appropriate expression vector.

To express the antigenic determinant for eliciting the immune response, the synthesized DNA fragments conforming to the above designs, were inserted into the eukaryotic expression or prokaryotic expression vectors by the standard recombinant DNA techniques known in the art.

**Inserting the DNA fragment into the eukaryotic expression vector for making DNA vaccine**

A number of eukaryotic vectors are available for making DNA vaccine, from commercial and academic sources. The regulatory/control sequences will depend on the host being treated and the type of preparation used. Thus, if the host’s endogenous transcription and translation machinery will be used to express the proteins, the control or regulatory sequences compatible with the particular host will be utilized. In this regard, several promoters for use in mammalian systems are known in the art and include, but are not limited to, promoters derived from SV40, CMV, HSV, RSV, MMTV, among others. In the present work, the eukaryotic expression vector based on a strong cytomegalovirus (CMV) promoter, was used. The vector also contained cytomegalovirus intron A and BGH derived polyadenylation sequences for regulating the expression. The DNA fragment was cloned into the expression vector by known techniques in the art like restriction digestion, ligation, etc. Fig. 1 shows a representative, though not unique, schematic construct of eukaryotic expression vector, which contains the cloned DNA fragment having nucleotide sequences as per SEQ. ID. No.1 or 2. The DNA conforming to the above vaccine design can be prepared in large amounts and with high fidelity and accuracy using a bacterial host, as described hereunder.

**Process for the extraction and purification of plasmid DNA using a bacterial host**

A large number of bacterial hosts with low or no endonuclease activity are known in the art for the propagation, amplification, isolation and purification of plasmid DNA. These include, but not limited to, *Escherichia coli* (E. coli) strains DH1, DH5α, C600, XL1-Blue. The
present invention employed *E. coli* strain DH5α for the extraction and purification of plasmid DNA, the detailed process of which comprising of:

(a) Growing the *E. coli* strain DH5α carrying the recombinant plasmid in 1 liter of Luria Broth media (10 g/l tryptone, 5 g/l yeast extract and 5 g/l sodium chloride) containing 50 mg/l kanamycin, at 37°C temperature and 200 rpm, on a rotary shaker.

(b) Harvesting the cells after 16 h of growth. Collecting the cells by centrifugation at 6000 X g for 10 min at 25 °C in a laboratory centrifuge.

(c) Re-suspending the cell pellet in 60 ml of re-suspension buffer (50 mM Tris, 10 mM EDTA, pH 8.0).

(d) Adding 60 ml of lysis buffer (0.2 N NaOH and 1% SDS) to the cell re-suspension and mixing gently. Incubating the mixture for 15 min at room temperature.

(e) Adding 60 ml of 3M potassium acetate solution (pH 5.5) and mixing the contents gently by inverting the tube 4-5 times. Incubating the mixture at room temperature for 15 min.

(f) Centrifuging the mixture at 12,000 X g for 20 min at 25°C. Collecting the clear supernatant and adding 0.6 volume of iso-propyl alcohol. Incubating the mixture at room temperature for 20 min.

(g) Centrifuging the contents at 12,000 X g for 20 min at 25°C. Discarding the supernatant.

(h) Rinsing the pellet with 5 ml of 70% ethanol, air-drying and re-suspending in 20 ml of TE (10 mM Tris, 1mM EDTA, pH 8.0) buffer.

(i) Adding 20 ml of ice-cold 5M LiCl, mixing well and centrifuging at 12,000 X g for 10 min at 4°C.

(j) Discarding the pellet and adding equal volume of iso-propyl alcohol to the supernatant. Incubating the mixture at room temperature for 20 min.

(k) Centrifuging the contents at 12,000 X g for 20 min at 25°C. Discarding the supernatant.
Rinsing the pellet with 5 ml of 70% ethanol, air-drying, and re-suspending in 8 ml of TE buffer.

Adding RNase solution to a final concentration of 100 µg/ml, and incubating the mixture at 37°C for 45 min.

Extracting the contents with phenol: chloroform: iso-amyl alcohol (25:24:1), twice, and with chloroform: iso-amyl alcohol (24:1), twice, at 4°C.

Adding 2 ml of 10 M ammonium acetate and 20 ml of ethanol. Centrifuging the contents at 12,000Xg for 20 min at 25°C.

Rinsing the DNA pellet with 5 ml of 70% ethanol, air-drying, and re-suspending in 1 ml of sterile water.

This corresponds to a pure preparation of the plasmid DNA, for use as a vaccine. The ingredients mentioned in the said process can be varied in the range of 10% for obtaining similar level of purity and yield of the plasmid DNA.

The purity-check and quantifications of isolated plasmid DNA can be done by agarose gel-electrophoresis and spectrophotometric absorption. The ratios of the absorbance at 260 nm and 280 nm wavelength, of the DNA samples, were observed in the range of 1.8-1.9, which is a standard for a good plasmid DNA preparation.

Inserting the DNA fragment into the prokaryotic expression vector for making protein vaccine

A number of prokaryotic vectors are available for making recombinant proteins, from commercial and academic sources. The regulatory/control sequences will depend on the host being treated and the type of preparation used. Thus, if the host’s endogenous transcription and translation machinery will be used to express the proteins, the control or regulatory sequences compatible with the particular host will be utilized. In this regard, several promoters for use in bacterial systems are known in the art and include, but are not limited to; T7, T5, tac, lac, pH, ΛP, among others. In the present work, the prokaryotic expression vector based on a strong T7 promoter, was used. The DNA fragment was cloned into the expression vector by known techniques in the art like restriction digestion, ligation, etc. Fig. 2 shows a representative, though not unique, schematic construct of prokaryotic expression...
vector, which contains the cloned DNA fragment having nucleotide sequences as per SEQ. ID. No.1 or 2.

The recombinant protein conforming to the above vaccine design can be prepared in large amounts and with high fidelity and accuracy using a bacterial host, as described hereunder.

**Process for the extraction and purification of recombinant protein using a bacterial host**

A large number of bacterial hosts with low or no protease activity are known in the art for extraction and purification of recombinant proteins. These include, but not limited to, *Escherichia coli* strains BL21, BL21(DE3), BL21(DE3)pLys, TG1, XL1-Blue. The present invention employed *E. coli* strain BL21(DE3) for the extraction and purification of plasmid DNA, the detailed process of which comprising of:

(a) Growing the *E. coli* strain BL21(DE3)pLys carrying the recombinant plasmid in 50 ml of Luria Broth media (10 g/l tryptone, 5 g/l yeast extract and 5 g/l sodium chloride) containing 100 mg/l ampicillin and 35 mg/l chloroamphenicol, at 37°C temperature and 200 rpm, over night on a rotary shaker.

(b) Transferring the grown culture in 1 liter of Luria Broth media containing 100 mg/l ampicillin and 35 mg/l chloroamphenicol, and growing further for 2-3 h at 37°C temperature and 200 rpm, on a rotary shaker.

(c) Inducing the said culture with 1 mM IPTG and allowing it to grow further.

(d) Harvesting the cells after 4-6 h of growth. Collecting the cells by centrifugation at 6000 X g for 15 min at 25 °C in a laboratory centrifuge.

(e) Re-suspending the cell pellet in 50 ml of re-suspension buffer (100 mM Tris pH 8.0, 10 mM EDTA, 100mM NaCl, 1mM PMSF).

(f) Disrupting the cells by sonication. Centrifuging the contents at 10,000 X g for 30 min at 4 °C and collecting the pellet.

(g) Washing the pellet containing inclusion bodies extensively with re-suspension buffer containing 1% deoxycholate and then with Milli Q water.
(h) Solubilizing the inclusion bodies by stirring for 45-60 minutes in 100 ml of denaturant buffer (100 mM NaH₂PO₄, 500 mM NaCl, 10 mM Tris, 8 M Urea, pH 8.0) and centrifuging the solution at 10,000 X g for 30 min at 25°C.

(i) Collecting the supernatant and loading onto a column packed with Ni-NTA equilibrated with the denaturant buffer.

(j) Washing the column with 80 ml washing buffer A (100 mM NaH₂PO₄, 500 mM NaCl, 10 mM Tris, 6 M Urea, pH 6.5) and 120 ml of washing buffer B (100 mM NaH₂PO₄, 500 mM NaCl, 10 mM Tris, 6 M Urea, pH 5.9).

(k) Collecting the recombinant protein with 100 ml of elution buffer (100 mM NaH₂PO₄, 500 mM NaCl, 10 mM Tris, 2 M Urea, pH 4.2).

(l) Refolding the recombinant protein by dialyzing urea out slowly at 4 °C.

(m) Concentrating the re-folded protein solution in a pressurized stirred cell fixed with ultra-filtration membrane.

The above-mentioned preparation corresponds to a pure preparation of the recombinant protein for use as a vaccine. The ingredients mentioned in the said process can be varied in the range of 10% for obtaining similar level of purity and yield of the protein.

The purity-check of the isolated protein can be done by SDS-PAGE and Western Blot analysis. Quantification of the protein can be done by protein estimation methods like Lowry, Bradford, Bi-cintronic acid assay methods.

**Administering the DNA vaccine**

The plasmid DNA can be administered as vaccine, alone or along with a compatible adjuvant. DNA encapsulated Microparticles of poly-lactic-co-glycolic acid (PLGA) copolymer, can also be used for injection. The recipients can be injected with DNA vaccine through any parenteral route, e.g., intramuscular, intradermal, subcutaneous, intranasal, oral routes.

It can also be administered by particle bombardment using a gene gun, or through a cationic transfection lipid. Muscle is a useful tissue for the delivery and expression of plasmid-coded genes because mammals have a proportionately large muscle mass, which can be readily accessed by direct injection through skin. A comparatively large dose of plasmid DNA can be
deposited into muscle by multiple and/or repetitive injections. Multiple injections can also be used for therapy over extended periods of time.

Present invention involved free plasmid DNA injection without any carrier. It was injected intramuscularly and it resulted in eliciting an immune response. Two or more injections at a dose of 100 μg each, given at about 15 days interval, were sufficient to generate anti-LHRH antibodies. The dose amount can be varied from 20 μg to 500 μg, each generating equal and sufficient response, causing a decline of testosterone to castration levels along with reduction in the prostate size.

**Administering the proteinic vaccine**

The said proteinic vaccine can be mixed with alum, sodium phthalyl lipopolysaccharide (SPLPS) or with other commercially available adjuvants. It can also be delivered as Microparticles of poly lactide, poly glycolide or poly-lactic-co-glycolic acid (PLGA) copolymer, and Nano particles of calcium phosphate. For experimental purposes and with the idea of demonstrating the proof of concept in the ability of the invented sequences to cause reduction of testosterone to castration level and bring about atrophy of the prostate, the vaccine was given intramuscularly to rats with Freund’s Complete Adjuvant, followed by a booster after 15 days with Freund’s Incomplete Adjuvant. The dose amount was varied in the range 20-500 μg per animal. Each animal elicited immune response in terms of anti-LHRH antibodies causing a decline of testosterone to castration levels along with reduction in the prostate size.
### Table 1. Prostate weights normalized with respect to the body weights in animals immunized with anti-LHRH proteinic vaccine as described in the text

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Anti-LHRH antibody titre (OD$_{492}$)</th>
<th>gm Prostate wt /100 gm Body wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control A</td>
<td>0.000</td>
<td>0.223</td>
</tr>
<tr>
<td>Control B</td>
<td>0.000</td>
<td>0.224</td>
</tr>
<tr>
<td>Control C (FCA)</td>
<td>0.000</td>
<td>0.219</td>
</tr>
<tr>
<td>Control D (FCA)</td>
<td>0.000</td>
<td>0.226</td>
</tr>
<tr>
<td>Animal 1</td>
<td>0.285</td>
<td>0.011</td>
</tr>
<tr>
<td>Animal 2</td>
<td>0.172</td>
<td>0.096</td>
</tr>
<tr>
<td>Animal 3</td>
<td>0.105</td>
<td>0.013</td>
</tr>
<tr>
<td>Animal 4</td>
<td>0.271</td>
<td>0.072</td>
</tr>
<tr>
<td>Animal 5</td>
<td>0.357</td>
<td>0.016</td>
</tr>
<tr>
<td>Animal 6</td>
<td>0.141</td>
<td>0.060</td>
</tr>
<tr>
<td>Animal 7</td>
<td>0.162</td>
<td>0.018</td>
</tr>
<tr>
<td>Animal 8</td>
<td>0.254</td>
<td>0.010</td>
</tr>
</tbody>
</table>

- The invention is further illustrated with reference to the following examples and should not be construed to limit the scope of the present invention.

**Example 1**

The DNA fragments with nucleotide sequences as described in the text were cloned into a eukaryotic expression vector. This recombinant expression vector was used to transform a
bacterial host, and then plasmid DNA isolated and purified for use as DNA vaccine. DNA vaccine was injected intramuscularly twice at 30 days interval, to male adult rats at a dose of 100 μg. The efficacy of the vaccine was determined in terms anti-LHRH antibody production, which was quantified by ELISA technique using LHRH as antigen. The vaccine is competent to produce antibodies measurable in blood, which are reactive with LHRH. The antibody response was produced in all animals in which immunization was done, irrespective of the genetic background of the mice or rats. Fig. 3 gives the results of antibody titres generated by DNA vaccines containing DNA fragments having nucleotide sequences as per SEQ. ID. No. 1 and 2.

Each one of the four animals in each group produced antibodies, the titres of which were rising even on 79th day of observation post immunization. The antibodies started to appear soon after immunization and were measurable even in the first bleed taken on day 23. Though individual variability is an inherent trait of immune response, every animal was positive for antibody response with titres varying from animal to animal as is normally expected in immune response. The antibody titres in each rat were higher than those required for exercising biological effect, which is evidenced by the data on testosterone levels, which declined in tune with emergence of antibodies. Testosterone levels can be determined using commercially available kits (e.g., cat no. RE 52151, IBL, USA). Fig. 4 shows declining testosterone levels perceptively with the production of the antibodies.

Example 2

The DNA fragments with nucleotide sequences as described in the text were cloned into a prokaryotic expression vector. This recombinant expression vector was used to transform a bacterial host, and then recombinant protein isolated and purified for use as proteinic vaccine. Proteinic vaccine mixed with Freund’s Complete Adjuvant (FCA), was injected intramuscularly, to male adult rats at a dose of 100 μg. Two booster doses were given along with Freund’s Incomplete Adjuvant, at 15 days interval. The efficacy of the vaccine was determined in terms anti-LHRH antibody production, which was quantified by ELISA technique using LHRH as antigen. The vaccine is competent to produce antibodies circulating in blood, which are reactive with LHRH. Fig. 5 gives the results of antibody titres generated.
by proteinic vaccines containing DNA fragments having nucleotide sequences as per SEQ. ID. No. 1 and 2.

Each one of the four animals in each group produced antibodies. The antibody titres in each rat were higher than those required for exercising biological effect, which is evidenced by the data on testosterone levels, which declined in tune with emergence of antibodies. Fig. 6 shows declining testosterone levels perceptively with the production of the antibodies. The biological effect of immunization on prostate size was determined by removing the prostate lobes surgically, the weights of which were significantly lower than those of control rats, which were either not immunized or immunized with FCA alone (Table 1). Further confirmation of the atrophy of the prostate was obtained by the histology of the extirpated prostate lobe as is evident from a representative figure (Fig. 7).

The above-mentioned examples suggest that immunization with the invented vaccines, are akin to surgical orchiectomy and interestingly a much more convenient alternative in patients suffering from prostate hypertrophy or prostate cancer.

**Results and advantages of the invention**

This invention provides the nucleotide sequence of DNA fragments comprising SEQ. ID. No. 1 or SEQ. ID. No. 2, and their insertion into a prokaryotic and a eukaryotic expression vector, for making a proteinic and DNA vaccine, respectively. The advantages associated with this invention comprise the following:

(a) The invention provides an immunological process to reduce prostate size without surgery in patients suffering from benign prostate enlargement. It will be a convenient approach avoiding hospitalization costs particularly beneficial to those patients, in whom surgery is hazardous for other medical reasons.

(b) The invention provides products and processes for generating antibodies competent to react with native LHRH/GnRH to reduce testosterone levels to castration level without orchiectomy.

(c) The invention provides products and processes for the treatment of prostate cancer, dependent on LHRH/GnRH and testosterone in mammals like humans.

(d) The invention provides products and processes wherein the vaccines can be used in place of costly LHRH agonists and antagonists which are used in
clinical practice to block the action of LHRH hormone in the therapy of carcinoma of prostate.

(e) The invention provides a method wherein the administration of the vaccine in the form of DNA, does not need any oil based adjuvant such as squalene, Arlacel A, MDP, etc., which could induce undesirable side effects of local granuloma and tissue reactivity.

(f) The invention provides products and processes wherein the vaccines can be used in other sex-steroid hormone dependent diseases like breast cancer in women.

(g) The invention provides products and processes wherein the vaccines can be used for reversible control of fertility and hormone dependent manifestations such as estrus of female companion animals like dogs and cats.

(h) The invention provides products and processes wherein the vaccines can be used to block the testosterone production to improve the quality of meat in animals raised for this purpose like pigs, rams, etc.

References


CLAIMS

1. An oligonucleotide sequence of SEQ ID No. 1 useful for preparing a recombinant anti-LHRH DNA vaccine.

2. An oligonucleotide sequence as claimed in claim 1, wherein the said SEQ ID No. 1 expressing corresponding polypeptide sequence of SEQ ID No. 3 useful as a recombinant anti-LHRH proteinic vaccine.

3. A polypeptide sequence of SEQ ID No. 3 useful for preparing a recombinant anti-LHRH proteinic vaccine.

4. An oligonucleotide sequence of SEQ ID Nos. 2 useful for preparing a recombinant anti-LHRH DNA vaccine.

5. An oligonucleotide sequence as claimed in claim 4, wherein the said SEQ ID No. 2 expressing corresponding polypeptide sequence of SEQ ID No. 4 useful as a recombinant anti-LHRH proteinic vaccine.

6. A polypeptide sequence of SEQ ID No. 4 useful for preparing a recombinant anti-LHRH proteinic vaccine.

7. A method of obtaining purified recombinant anti-LHRH DNA vaccine using oligonucleotide sequence of SEQ ID No. 1 or 2, said method comprising the steps of:
   a) designing an oligonucleotide sequence of SEQ ID No. 1 or 2,
   b) inserting the said sequence into a eukaryotic expression vector comprising an appropriate promoter,
   c) replicating the expression vector containing the oligonucleotide sequence of SEQ ID No.1 or 2 in an endonuclease-free bacterial host,
   d) extracting plasmid DNA from the bacterial host of step (c), and
   e) purifying plasmid DNA to obtain the DNA vaccine.

8. A method as claimed in claim 7, wherein the promoter is selected from a group comprising SV40, CMV, HSV, RSV, and MMTV.

9. A method as claimed in claim 7, wherein the bacterial host is selected from a group comprising *E. coli* DH1, DH5alpha, C600, and XL1-Blue.
10. A method as claimed in claim 7, wherein the vector is selected from a group comprising VR1012, VR1020, pRc/CMV, pUMVC7, and pVAC.

11. A method of obtaining recombinant anti-LHRH proteinic vaccine using polypeptide sequence of SEQ ID No. 3 or 4, said method comprising the steps of:
   a) designing an oligonucleotide sequence of SEQ ID No. 1 or 2,
   b) inserting the said sequence into a prokaryotic expression vector comprising an appropriate promoter,
   c) replicating the expression vector containing the DNA sequences of SEQ ID No. 1 or 2 in a protease-free bacterial host,
   d) extracting recombinant protein from the bacterial host of step (c), and
   e) purifying and refolding the recombinant proteins to obtain the proteinic vaccine having polypeptide sequence of SEQ ID No. 3 or 4.

12. A method as claimed in claim 11, wherein the promoter is selected from a group comprising T7, T5, tac, lac, pH, and $\lambda p_L$.

13. A method as claimed in claim 11, wherein the bacterial host is selected from a group comprising E. coli strains of BL21, BL21(DE3), BL21(DE3)pLys, TG1, and XL1-Blue.

14. A method as claimed in claim 11, wherein the vector is selected from a group comprising pRSET, pET, pQE, and pSE420.

15. A vaccine composition comprising plasmid DNA containing oligonucleotide sequence of SEQ ID No. 1 optionally along with an adjuvant and/or delivery system useful as a recombinant anti-LHRH DNA vaccine.

16. A vaccine composition comprising protein of polypeptide sequence of SEQ ID No. 3 optionally along with an adjuvant and/or delivery system useful as a recombinant anti-LHRH proteinic vaccine.

17. A vaccine composition comprising plasmid DNA containing oligonucleotide sequence of SEQ ID No. 2 optionally along with an adjuvant and/or delivery system useful as a recombinant anti-LHRH DNA vaccine.
18. A vaccine composition comprising protein of polypeptide sequence of SEQ ID No. 4 optionally along with an adjuvant and/or delivery system useful as a recombinant antilHRH proteinic vaccine.

19. A safe, economical, and effective method of reducing levels of sex steroid hormones by eliciting an effective antibody response against Luteinizing hormone Releasing Hormone (LHRH) in mammals using either DNA vaccine containing oligonucleotide sequence of SEQ ID No. 1 or 2, or proteinic vaccine having polypeptide sequence of SEQ ID No. 3 or 4, said method comprising steps of administering said DNA or proteinic vaccine optionally along with an adjuvant and/or a delivery system to the mammal through parenteral route, and thereby reducing levels of sex steroid hormones.

20. A method as claimed in claim 19, wherein the delivery system is selected from a group comprising Microparticles of poly lactide, poly glycolide or poly-lactic-co-glycolic acid (PLGA) copolymer, Nano particles of calcium phosphate, Cationic transfection lipids, and Gene gun.

21. A method as claimed in claim 19, wherein the sex steroid hormones are testosterone in males, and progesterone and estrogen in females.

22. A method as claimed in claim 19, wherein the said method is used for controlling and/or treating the sex-steroid hormone dependent cancers.

23. A method as claimed in claim 19, wherein said method brings down the levels of testosterone to castration levels without surgical removal of testes.

24. A method as claimed in claim 19, wherein said method leads to atrophy of the enlarged prostate.

25. A method as claimed in claim 19, wherein the said method is employed in the treatment of prostate cancer.

26. A method as claimed in claim 19, wherein said method is employed to suppress heat/estrus in female companion animals.

27. A method as claimed in claim 19, wherein said method is employed to improve quality of meat of animals.
28. A method as claimed in claim 19, wherein the said method is employed to treat sex-
hormones dependent breast cancers in women.

29. A method as claimed in claim 19, wherein the said method is used for reversible
control of fertility in male animals.

30. A method as claimed in claim 19, wherein parenteral route comprises intramuscular,
intradermal, subcutaneous, intranasal, and oral route.

31. A method as claimed in claim 19, wherein multiple injections of the vaccine can be
given over an extended periods of time.

32. A method as claimed in claim 19, wherein dosage of both DNA and proteinic vaccine
is ranging between 20 to 500 μg per dose once or twice in a month.

33. A method as claimed in claim 19, wherein the adjuvants are selected from a group
comprising alum, sodium phthalyl lipopolysaccharide (SPLPS), Freund’s complete
adjuvant/Incomplete Freund’s adjuvant, and other commercially available adjuvants.

34. An oligonucleotide sequence of SEQ ID No. 1 substantially as hereindescribed and
illustrated therein.

35. A safe, economical, and effective method of reducing levels of sex steroid hormones
substantially as hereindescribed and illustrated therein.
VR1012-L
5400 bps

FIG. 1
FIG. 3

DNA vaccine (1)

DNA vaccine (2)

Days post immunization

Anti LHRH antibody titers (OD₄₀₂)

animal no. 1
animal no. 2
animal no. 3
animal no. 4
animal no. 5
animal no. 6
animal no. 7
animal no. 8
FIG. 4
FIG. 5

![Graph showing anti-LHRH antibody titers over days post immunization for different animals and vaccine types.]

- Animal no. 1
- Animal no. 2
- Animal no. 3
- Animal no. 4
- Animal no. 5
- Animal no. 6
- Animal no. 7
- Animal no. 8

Proteinic vaccine (1) vs Proteinic vaccine (2)
FIG. 6

[Graph showing testosterone levels over days post immunization for different animals and two proteinic vaccines.]