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Review Article

Recent advances in immunomodulation of reproduction*

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ABSTRACT

Modulation of reproductive functions through immunological approach in livestock is fairly a recent development. Gonadal functions can be suppressed through the use of antifertility vaccine developed against reproductive hormones and gamete antigens (Sperm and zona pellucida of ovum). This concept has been utilizied for immunosterilization of animals. Ovulation rate and reproductive efficiency on the other hand, may be increased through the use of fecundity vaccine. Immunization against inhibin has shown promising results for increasing ovulation rate and litter size. Immunomodulation of uterine defense is another area currently being under intensive investigation. Immunomodulators may be considered as an alternative therapeutic agent in clinical and subclinical uterine infections. Trophoblastic protein(s) or interferon purified recently in some of the animal species has potential application in regulation of fertility.

Key words : Hormone vaccine, immunomodulator, inhibin, trophoblastic protein

Fertility management is a global issue. Regulation of reproduction by immune intervention is fairly a recent development. Immunization by raising antibodies against reproductive hormones and antigens have been used to develop experimental model for fundamental research in reproduction and to explore potential for augmenting reproductive efficiency in livestock. Amongst methods which have emerged as being of use to modulate reproductive function are immunization against steroid and peptide hormones (Garza *et al.*, 1986; Terqui *et al.*, 1995). Concept of immunosterilization for population control and better management of recretional animals has also been given (Gaubau *et al.*, 1989; Hoskinson *et al.*, 1990). Passive immunization against PMSG to prevent ovarian over stimulation in superovulated animals has yielded good results (Gonzalez *et al.*, 1995). Possible application of immunomodulators and or cytokines for increasing uterine defense in case of microbial invasions and to monitor embryonic and fetal development are currently under intensive investigation. In the present paper, advances in immunoregulation of reproduction and their application in enchancing animal production have been discussed.

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Reproductive immune system : Immune system maintains relatively a sterile environment in reproductive tract. Female reproductive tract, like respiratory and gastrointestinal tracts is continuous with external environement of the animal. The tract is repeatedly subjected to invasion by microbial organisms. Contaminants may enter into female reproductive tract during insemination/natural service, at calving and during post partum period, particularly if sanitary conditions are not maintained. These micro organisms are rapidly removed as a result of the clearing mechanism of immune system. However, sometimes the immune defense mechanism in the reproductive tract is not sufficient, which may lead to inertility and sterility.

Immune system provides protection through various cells, viz., phagocytes (engulf and kill the antigen-microbes), B-lymphocytes which produce antibodies against invading microbes and T-lymphocytes responsible for secretion of cytokines to stimulate macrophages. These cells are organized in specialized tissues such as the thymus, spleen, lymph nodes, blood, mucosa and in various organs, especially those coming in contact with external environment. Two types of immune responses i.e., innate and acquired occur following the entry of foreign organism in the body. The most immediate response to invasion of foreign antigen

most immediate response to invasion of foreign antigen is mediated by phagocytic cells of the innate immune system. Macrophages and neutrophils engulf foreign particles and microbes (phagocytosis). This process can be stimulated by other components of immune system. Cytokines (regulatory molecules/proteins) produced by T-lymphyocytes can activate phagocytes and make them more efficient. Acquired immune system display the immune response through T and B lymphocytes. Both T and B cells express receptors on their cell surfaces that are specific for a particular antigen. Lymphocytes when recognize an antigen, undergo activation and proliferation. This results in formation of antibodies and memory cells. Immune mechanism involving T-lymphocytes is cell mediated while B-lymphocytes subscribe to humoral immunity. B cells destroy the microbes by secreting specific antibodies that circulate in the blood. Purified or monoclonal antibodies, through passive immunization may be used for immediate neutralization of the pathogen or specific hormone. T-cells are of two types i.e. cytotoxic T-cells and helper T-cells. They provide immunity by killing the infected cells and by helping the B cells through their secretion i.e. cytokines (Interleukins, interferon, tumor necrosis factor). Cytokines, in broader sense, are a variety of proteins which regulate the immune response by signalling between the cells.

Cervix is a major physical barrier to prevent the entry of organisms further into the reproductive tract. Cervical constriction in luteal phase and plugging during pregnancy checks the entry of micro organisms into the uterus. Lactoferrin present in cervical mucus is known to inhibit bacterial growth. In addition to physical barrier, neutrophils, T and B-lymphocytes and other cells of immune system help to maintain the reproductive tract sterile.

Immune defense of reproductive tract depends on the stage of the oestrus cycle. Steroid hormones regulate neutorphil function in the uterus. Estradiol-17β, at estrus, facilitate removal of micro organism while progesterone in luteal phase favours establishment of uterine infection (Washburn *et al.*, 1982; Asbury and Hensen, 1987). Local production of antibody in the reproductive tract is also regulated by ovarian steriod hormones. Concentration of IgA in uterine fluid has been reported to be higher at estrus than during luteal phase (Le Blanc *et al.*, 1988). Immune defense operating at estrus, helps in clearing micro-organisms entry into the uterus during mating or A.I. and removal of excessive spermatozoa to avoid development of immunity in the females against sperms. Despite the presence of this mechanism to prevent immune response to sperms, females sometimes develop anti-sperm antibodies. Anti-sperm antibodies have been recovered from the reproductive tract in cattle. This leads to immuno-infertility. Anti-sperm antibodies in female genital tract may lead to impaired cervical mucus production, aggultination, inactivation of acrosomal enzymes and increased phagocytosis of spermatozoa. Antibodies against zona antigens may block the fertilization by agglutination of egg or blocking sperm attachment. The incidence of antisperm antibodies in female farm animals has not been accurately established. It is also not well known that to what extent, animals with naturally occurring antisperm antibodies, are infertile. Till date no specific treatment is available except breeding rest. Changing of sire, administration of immunosupressant, intra-uterine deposition of killed spermatozoa prior to A.I. may be recommended to get rid of immuno-infertility in livestock.

Mother-foetus immunogenic relationship : Since half of the genome of the foetus is paternally derived which is foreign to the mother, fetus is considered a different individual (allograft) and it should be rejected by the mother. But unlike the immunologic destruction of allograft, in other parts of body, the foetus is not rejected until parturition. This has puzzled immunologists and led to many theories to explain the existence of foetus in the uterus. The nature of immune regulation mechanism is controversial. Trophoblast acts as a barrier, preventing entry of maternal lymphocytes to the fetus. This protection from maternal immunologic attack might be due to trophoblastic cell surface structure (sialomucin) and synthesis of factors that render it insensitive to an antibody or cell mediated immunologic lysis. The immunological rejection of bovine conceptus is blocked because of reduced expression of major histocompatability antigen (MHC) on the surface of the conceptus. An intra-uterine environment is created during pregnancy in which elevated progesterone secreted by maternal tissues and lymphocyte inhibitory molecules by the trophoblasts, reduce immuno reactivity. Specific changes in the functional activity

of maternal lymphyocytes population during pregnancy lead to 1) redirection of immune response 2) transient tolerance to conceptus antigens 3) activation of specific lymphocyte population involved in immuno suppression 4) release of cytokines that stimulate placental functions (Hansen, 1997).

Immunization against reproductive hormones : Reproductive functions in human and animals are largely controlled by hormone signalling beween the brain, pituitary and gonads. This signalling and recognition systems can potentially be targeted by immunological approach.Antibodies can be directed to circulating hormones. Antibodies can be targeted to surface antigens which are involved in gamete interactions during fertilization. Hypothalamus (higher brain centre) secretes gonadotrophin releasing hormone (GnRH/LHRH) to stimulate anterior pituitrary to secrete follicle stimulating hormone (FSH) and luteinizing hormone (LH) which together control testicular and ovarian functions. The gonads secrete a range of steroid and protein hormones which in turn regulate GnRH, FSH, LH secretion through feed back mechanism. The gonadal hormones help in maintenance and development of accessory sex glands, secondary sexual characteristics and sexual behaviour. Hormones are transported in the general circulation where they are readily accessible to antibodies. Therefore, antibodies specific for particular hormone consequently block its biological action. This concept has largely been substantiated to develop vaccine against reproductive hormones. Gondadal steroids and LHRH are non immunogenic, therefore, require conjugation to antigenic carrier proteins for immunization (Hoskinson *et al.*, 1990). Gonadotrophic hormones (FSH/LH) are immunogenic and do not necessarily require conjugation. However, improvement in antibody production response has been observed when gonadotrophic hormones are conjugated to carrier proteins (Desilva *et al.*, 1986).

Suppression of gonadal functions-Antifertility vaccine

A. Immunization against gonadotrophins and gonadotrophin releasing hormone : Gonadotrophin (FSH/LH) and gonadotrophin releasing hormone (LH-RH) play a major role in the structural and functional maintenance of gonads. Immunization against LH/FSH has been used to study gametogenesis and steroidogenesis. FSH immunization may be used as contraceptive. Suppression of ovarian activity (Maturation of follicle and ovulation) following immunization against a bovine LH-ovalbumin conjugate has been reported in cattle (Desilva *et al.*, 1986; Grieger and Reeves, 1980), sheep (Roberts and Reeves, 1989) and monkeys (Thau *et al.*, 1987). Immunization against human chorionic gonadotrophin (hCG) has extensively been studied to develop contraceptive vaccine. This hormone has been of particular interest in women as it does not interfere with normal ovarian function (Talwar and Singh, 1988; Singh *et al.*, 1989).

Immunization against LH-RH is of particular interest in livestock where control of sexual behaviour as well as fertility is important. Immunological neutralisation of LH-RH blocks pituitary secretion of LH/FSH and results in gonadal quiescence. Recent studies have demonstrated potential application of LH-RH immunization as an alternative to surgical and chemical sterilization. Young bulls immunized against LH-RH, show a delay in the onset of puberty and sexually mature bulls undergo atrophy of testes and a decline in circulating testosterone to castrate levels (D, occhio, 1993). LHRH immunization delay puberty in heifers and they remain prepubertal while sexually mature animals revert back to a prepubertal conditions (Falvo *et al.*, 1986; McNeilly *et al.*, 1986; Johnson *et al.*, 1988). The nature of LH-RH immunization is reversible. This feature of immunization may be exploited to develop vaccine for situations where suppression of fertility is required only for a defined period.

B. Immunization against gamete surface antigen (Sperm and zona pellucida antigen) : Fertilization i.e. interaction between egg and sperm involves specific surface antigens present on the gamete of both sexes. These antigens have been potential target for contraceptive vaccine. Different types of antigens on the surface of sperm have been described. Immunization with such sperm antigen(s) either prevent fertilization or reduce survival of embryo in the uterus (Naz *et al.*, 1986). Immunization of males against sperm antigens also reduced the fertilization rate (Mahi-Brown *et al.*, 1990).

Zona pellucida is a glycoprotein membrane which surrounds mammalian egg. This is composed primarily of three glycoproteins i.e. ZP1, ZP2 and ZP3 (Yanagimachi, 1988). ZP glycoproteins are involved in sperm binding during sperm penetration through zona and has, therefore, been tergeted for contraceptive vaccine. Immunization against zona pellucida has been

successful in preventing pregnancy in animals (Kirkpatrick *et al.*, 1990).

Increase in ovulation rate-fecundity vaccine

A. Immunization against gondal steroid : Gonadal steroid hormones play an important role in sexual behaviour, function of accessary sex glands, establishment and maintenance of pregnancy. Reproductive consequences have been studied following immunization against androgen, estrogen and progesterone. Immunization against testosterone stimulates ovarian activity but it usually causes abnormal ovarian cycles (Price *et al.*, 1987). Androstenedione is another androgen used for immunological study. Immunization against androstenedione increases ovulation rate and fecundity in sheep and thus, can be used as fecundity vaccine (Boland and Crosby, 1993).

B. Immunization against inhibin : Inhibin, a glycoprotein hormone secreted by granulosa cells in the ovary plays an important role in FSH control through negative feed back mechanism. Inhibin suppresses secretion of FSH and thus regulates onset of oestrus (Ying, 1987). Administration of steroid free follicular fluid in cattle delays onset of estrus suggesting presence of inhibin (Law *et al.*, 1992; Singh *et al.*, 1997). Higher concentration of inhibin has been reported in buffalo follicular fluid (Palta *et al.*, 1995).

Immunization against inhibin has been suggested with a view that immunoneutralization of endogenous inhibin would enhance FSH secretion and cause an increase in ovulation rate and litter size. Initially, immunization against inhibin was done using crude or partially purified fraction of follicular fluid. Later on with the establishment of its amino acid sequence, recombinant vaccine was developed. An increase in ovulation rate in cattle using crude ovine follicular fluid (oFF) as immunogen has been observed by Cummins *et al.* (1986), Price *et al.* (1987). In sheep, on the other hand crude preparation of follicular fluid produced variable and transient increase in ovulation rate (Al-Obaidi *et al.*, 1987). Reliability of response increased with the purity of follicular fluid using immuno-affinity chromatography (Bindon *et al.*, 1988; O'shea *et al.*, 1989). Active immunization against synthetic fragment of porcine, ovine and bovine inhbin α-subunit conjugated to suitable carrier protein has resulted in 2-4 folds increase in ovulation rate (Wheaton *et al.*, 1992). Active immunization against various synthetic bovine or procine inhibin α-subunit fragment has also resulted in elevation of FSH level, increase in follicular number and ovulation rate (Glencross *et al.*, 1994). Immunization against α-subunit of recombinant human inhibin in goat produced high antibody titre, 3 times increase in ovulation rate and yield of embryo (Dietrich *et al.*, 1995). Passive immunization using follicular fluid and inhibin antisera also resulted increase in ovulation rate in most of the animal species (Kaneko *et al.*, 1993; Takedomi *et al.*, 1995; Kumar *et al.*, 1998).

C. Passive immunization : Use of anti PMSG serum : PMSG is a glycoprotein hormone, used for superovulation and recovery of large number of embryos in livestock. It has longer biological half life and, therefore, remains in circulation for a longer period and thus produces adverse effect on ovarian response and embryo production. Using anti-PMSG serum, ovarian over stimulation and secondary rise in estradiol level affecting viability of embroy can be prevented in cattle (Gonzalez *et al.*, 1994).

Modulation of uterine defense : An optimal uterine environment is one of the basic requirements for the viability of spermatozoa and development of embryo. The uterine defense mechanism normally prevents colonization of invading micro organism in the uterus. Severity of infection may overcome the uterine defense and lead to endometritis. Neutrophils, monocytes and tissue macrophages are the phagocytic cells involved in uterine defense. Immunoglobulins also play an important role in defense mechanism. Intrauterine antibiotic therapy has been in use for the treatment of endometritis and metritis. The major problem faced with antibiotic therapy is the frequent development of the bacterial resistance. Immunomodulators such as *E. coli* lypopolysaccharides (*E. coli*-LPS) and levamisole in smaller doses have been found effective in the treatment of endometritis (Agarwal and Tomar, 1998). *E. coli* - LPS has successfully been used as an immunomodulator, an alternative to antibiotics for the treatment of subclinical and clinical endometritis (Hussain and Daniel, 1992; Saini *et al.*, 1999). PMN extracts and GM-CSF increases uterine defense and may be used for the treatment of uterine infections (Cauto *et al.*, 1987; Cullor, 1987).

Immunomodulation during pregnancy : Trophoblast of sheep and cattle embryo secrete ovine and bovine trophoblastic protein (Interferon-t or IFN-t). These proteins are a type of cytokine responsible for maternal recognition of pregnancy and embryonic development (Stewart *et al.*, 1992). Trophoblast interferone acts locally within the uterus to prevent luteolysis and to promote progesteron secretion. This signals the mother for maintenance of conceptus. Such protein(s) are secreted before blastocyst attaches to endometrium. Therefore, these protein(s) may be used as potential fertility promoters, specially in subfertile or infertile animals. Bovine trophoblastic protein has been called as pregnancy specific protein (PSP). Such protein(s) have been isolated, partially purified and used for early diagnosis of pregnancy and foetal development in cattle (Dobson *et al.*, 1993 and Vasques *et al.*, 1995) and buffloes (Singh *et al.*, 2000).

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