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Popular Article

## A Review: Immunological Approaches In Animal Fertility

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### *Abstract*

Animal fertility is very important when we are concerned about dairy farmers, which can be modulated by some of immunological approaches. This approach uses antigen or antibody in required dose to manipulate fertility by increasing the innate immunological reactions. These approaches can be used for either immuno-enhancement or immuno-suppression with several hormones like GnRH, LH, Inhibin, Androstenedione, etc. As such hormones does not induce immunogenicity, for reliable production of antibodies, the peptide has to be first conjugated to a carrier immunogenic protein molecule. Some of the examples are like Inhibin vaccination, Fecundin® vaccine against androstenedione, Lymphocyte immunization therapy (LIT)- an effective anti-abortion method. Hormones like estrogen, PGF<sub>2α</sub>; E.coli Lipopolysaccharide, serum, plasma, hyper-immune plasma, polymorphonuclear (PMN) extracts, mycobacterial cell wall extract, etc are some of immunomodulators can be used as an alternative therapy for the treatment of uterine infections which activates the natural defense mechanisms in the uterus. In immuno-contraception GnRH vaccine, Porcine Zona Protein (PZP) vaccines are some of the examples. These are helpful in reducing the aggressive behavior of beef bulls and rams, reduces the boar taint and unexpected pregnancies, blocking the fertilization in different species by blocking the binding of zona pellucida and sperm membrane. Immunological approaches are helpful in both reducing and increasing fertility effectively.

### **Introduction**

#### **Animal fertility**

In female it is the desire and ability to mate, capacity to conceive, nourish the embryo, expel a normal fetus along with its membrane and have a potency to restore its normal physiology (Abraham,



2017). In male it is the desire and ability to mate, and produce right quantity and quality of spermatozoa which causes pregnancy in fertile female.

### **Immunological approaches**

It is the use of antigen or antibody, in required dose to manipulate fertility by increasing the innate immunological reactions. These can be used either to improve the fertility (Increase ovulation rate, to treat endometritis, against abortion, control hormone dependent cancer) or to reduce the fertility (Immuno-contraception, delay the estrus, maternal termination of pregnancy).

For normal fertility, hypothalamus releases GnRH, acts on anterior pituitary to release gonadotropins i.e, LH and FSH, which in females acts on ovary. FSH helps in follicular development and LH helps in ovulation. In males, LH acts on leydig cells and produces testosterone. Testosterone and FSH both acts on sertoli cells and helps in spermatogenesis. Any of this process if affected, impairs fertility.

Normally, when an antigen enters body it initiates an adaptive immunity, where helper T cells, B cells will differentiate, produces antibodies and cytokines which in turn causes lysis or phagocytosis of antigen. This same concept is used in vaccination process, to induce a mild adaptive immunity and protect animals from further infection. The same mechanism is targeted against hormones to improve or reduce fertility of animals as and when required. Simply injecting hormones will not initiate any immunogenicity. They need to be linked to carrier protein to enhance the antigenicity. Example- GnRH is conjugated to a carrier immunogenic protein molecule which enhances antigenicity of a self-protein. **Alum-precipitated beta-subunit of human chorionic gonadotropin (hCG)** chemically linked to **tetanus toxoid**. The **immune response** to a given antigen is **enhanced** by the inclusion of an **adjuvant**, (adjuvare, to help or aid) i.e., a substance that non-specifically stimulates an immune response (Alexander and Brewer, 1995).

### **History**

Scaramuzzi, Davidson and Van Look, published a pioneering report in **1977**, stating that active immunization against androstenedione increased ovulation rate in Welsh mountain ewes. In **1983**, a vaccine against androstenedione was released commercially as **Fecundin®**. In the **1980s**, the immunological approach for contraception as an alternative to surgical sterilization of animals gained momentum (Kirkpatrick *et al.*, 2011). Since the **1970s**, Zona pellucid (ZP) has drawn considerable attention as a promising target for immunocontraception. In the classic studies in 1980s and 90s, wild



horses were immunized with PZP vaccines. Immunological approaches mainly target on- GnRH, LH, FSH, hCG, Sperm antigen, zona pellucida of oocyte, trophoblast cell layer of blastocyst.

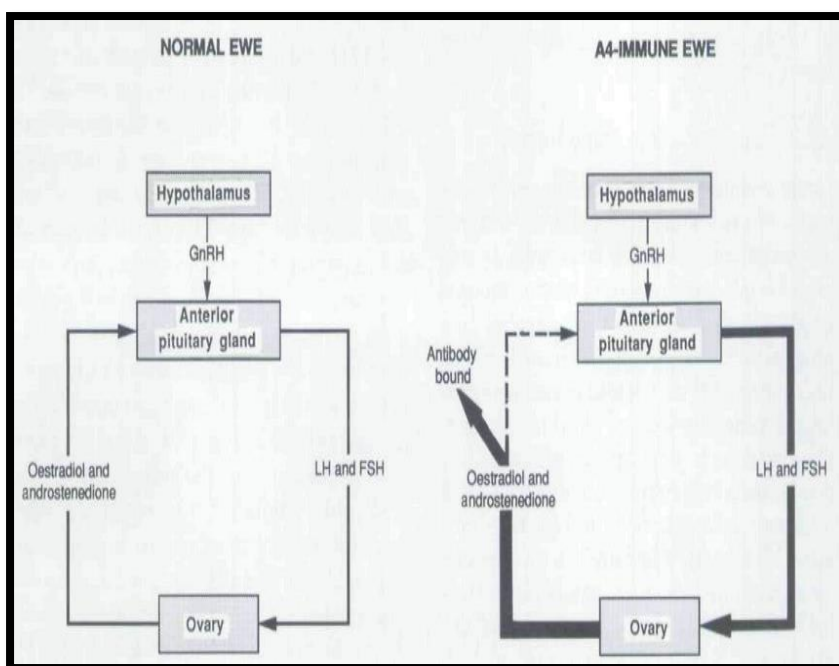
## Promotion Of Fertility

### 1. Inhibin immunization in sheep and cattle

Inhibin is a glycoprotein consisting  $\alpha$  and  $\beta$  subunit, which **inhibits** the synthesis or secretion of the pituitary gonadotrophins, preferentially FSH. The **ovarian follicle** is the major and the only **source of inhibin** in ovine and bovine. The idea to immunize sheep and cattle against inhibin came from the observation that the ovary of the **prolific Booroola Merino** was **deficient in bioactive ovarian inhibin**. It was postulated that the **elevated plasma FSH** concentrations and **exceptional ovulation rates** of the Booroola were a consequence of this **inhibin deficiency**. **Immunoneutralization of inhibin** leads to **elevated levels of FSH**, during follicular phase, **increases the number** of large, **ovulatory follicles**, with small peptides (e.g., porcine  $\alpha$  7-26) which **persist for more than a year** after two booster injections. Inhibin vaccination, **overcomes the variability in superovulation** for embryo transfer in sheep and cattle. Immunization of female Japanese quail with recombinant chicken inhibin protein has shown accelerated puberty and enhanced egg production (Moreau *et al.*, 1998).

### 2. Androstenedione immunization

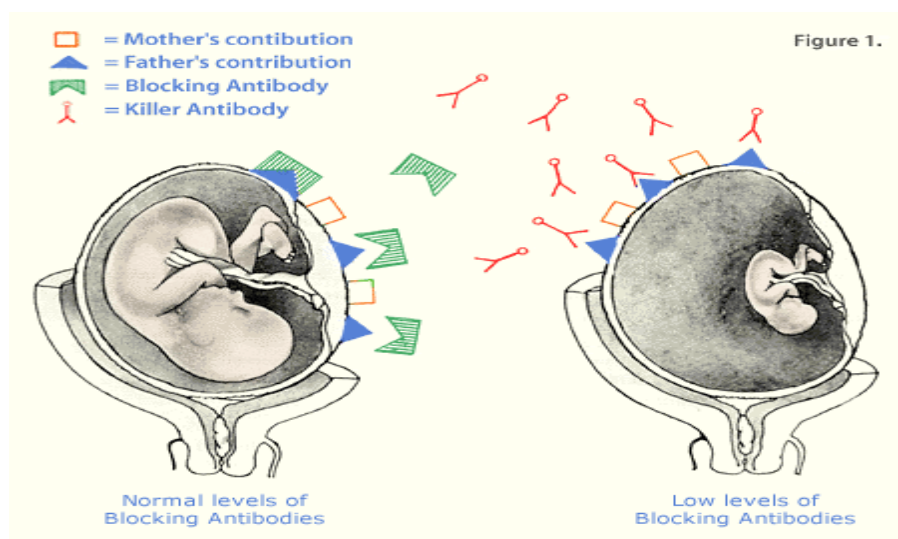
The release of LH and FSH from anterior pituitary acts on ovary, causes releases of estrogen and androstenedione which gives negative feedback on hypothalamus for releasing FSH. Thus when androstenedione immunization is done, this releases the antibodies against androstenedione and to some extent estrogen. These antibodies block the action of androstenedione and release of



FSH continues, which helps in increasing the ovulation rate. In **1983**, a vaccine against androstenedione was released commercially as **Fecundin®**.

### Lymphocyte Immunization Therapy (LIT)

In this therapy white blood cells from the prospective father are injected into the skin of the prospective mother, to prepare maternal immune system for pregnancy. Pregnancy tissues are product of both mothers and the father's genes. LIT assists the mother's immune system in **development of immunologic tolerance** to the genetically foreign pregnancy tissues. LIT is the treatment for those with **low Leukocyte antibodies**.



When a pregnant woman has a low LAT score (meaning: low Leukocyte antibodies), her antibodies do not recognize the protein from the father of the baby. An embryo's protein is half from the mother and half from the father. For these antibodies, the father's protein is considered foreign bodies. The antibodies "attack" these foreign bodies, thus resulting to pregnancy loss. With LIT, since the mother's antibodies seem "allergic" to the father's protein, white blood cells from the father will be injected to the mother. After several LIT, the LAT score is expected to be higher.

### 3. Immunomodulators to resolve endometritis in cow

Postpartum uterine infections result from uterine contamination with bacteria during parturition. Reproductive disorder is one of the main impediments in success of dairy husbandry and causes considerable magnitude of **economic losses** in livestock sector in India. Treatment of endometritis with parenteral or intra-uterine infusion of the antibacterial agents and antibiotics. They also markedly **destroy the phagocytic activity of polymorphonuclear leucocytes**, which are



responsible for maintaining non-specific uterine defense. So, there is urgent need to find out an **alternative therapy** for treatment of uterine infections by **activation of natural defence mechanism (UDM) in the uterus**. Under **normal** circumstances, **UDM** prevents invading bacteria from colonizing in the uterus but when this mechanism gets impaired or weekend, bacteria may colonize in the uterus and lead to endometritis. Endometritis is often self-limiting as infections are eliminated either by **contractions of the myometrium**, which forces the lochia to come out through the cervix or by **phagocytic activity of leucocytes** in the uterine fluids and endometrium.

### Therapy for endometritis

#### Hormones:

##### ➤ Estrogen

It increases the natural UDM-Through increasing the blood circulation (Leucocytic infiltration into the uterus), uterine contraction and mucus flow. But its **appreciable levels have been detected in milk** that may make it unfit for human consumption. Furthermore, the use of estrogen in higher doses could lead to the **development of cyst** in the ovaries and sudden drop in milk production.

##### ➤ Prostaglandin F2a analogues

**PGF2a** treatment shortens diestrus during which the uterus is under the effect of progesterone and hastens return to estrus during which the uterine defense mechanisms are more active, stimulates myometrial contraction, ideally makes it a good treatment for endometritis.

##### ➤ *E. coli* lipopolysaccharides (LPS)

The intra-uterine use of LPS acts as a **chemo attractant** to the PMN. It causes influx of serum protein and **immunoglobulin** to the uterus. *E. coli* LPS **effectively improved the stimulation of non-specific cellular immunity** to eliminate the endometritis in the cows and found **high pregnancy rate following intra-uterine infusion** of 100µg *E. coli* LPS in cows with endometritis and repeat breeding.

##### ➤ Serum, plasma or hyper-immune serum:

Addition of a small amount of serum to **uterine secretions** can increase the opsonizing capacity and enhances the **phagocytic ability** of PMNs significantly. In an experiment on mare, Jochle (1998) reported that 5 **uterine lavages with saline** solution, followed by an instillation of 120



ml **autologous plasma** with or without oxytocin took **2.7 days** to cure when the mares were experimentally infected with  $1 \times 10^9$ /ml *Strep. zooepidermicus* during estrus.

➤ **PMN extracts and their components**

Bovine neutrophil extract contains three small antibiotic peptides called **defensins**, which has significant bactericidal activity against both gram positive and negative bacteria, viruses. These PMNs could be utilized as **non-antibiotic therapy for endometritis**.

➤ **Mycobacterial Cell Wall Extracts (MCWE)**

**Mycolic acid**, which is a component of MCWE, has been shown to cause a **rapid and widespread influx of PMNs**, which is chiefly responsible for the clearance of uterine infection, by stimulating-  $TNF\alpha$ , Interferon  $\gamma$ , IL-6, IL-2 and Myeloperoxidase production and by suppressing IL-10 production. It is also reported that MCWE are effective by **intravenous or intrauterine** administration.

➤ **Leucotriene B 4 (LTB 4)**

An **arachidonic acid metabolite**, physiologically produced by stimulated granulocytes, macrophages and mast cells and plays an important role in neutrophil accumulation during inflammation. A single intrauterine treatment of 50 ml of 30 nmol/L solution increased the intra-uterine leucocyte count 5-10 times within 24 hrs.

➤ **Oyster Glycogen (OG)**

A single intra-uterine infusion of 500 mg OG in cows stimulated large no. of PMN cells migration into uterine lumen and up to 90% of them are neutrophils, clears bacterial infection and improves conception rate.

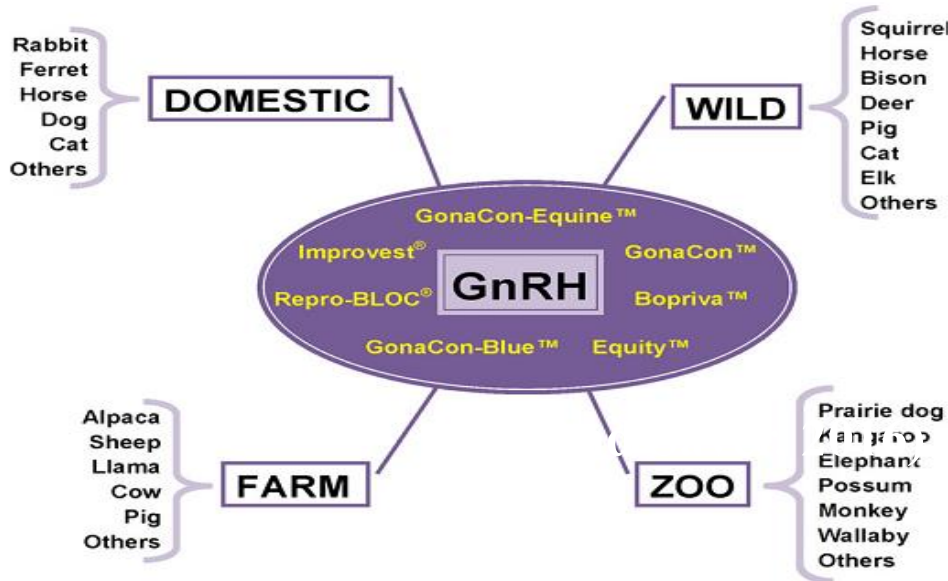
## **Decrease In Fertility (Immunocontraception)**

➤ **Immunization against GnRH**

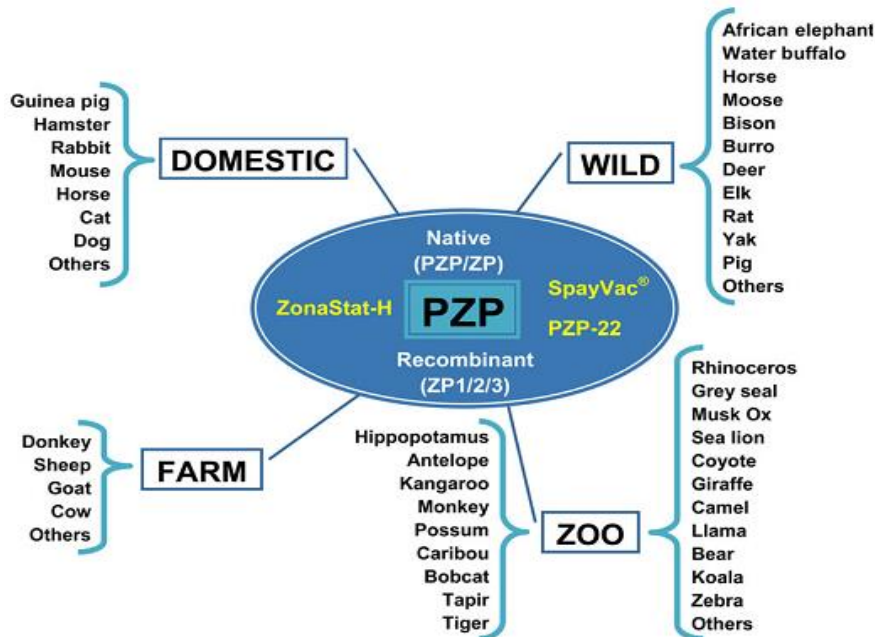
Uncastrated bulls and rams are aggressive, fight with each other frequently, injuring breeders and leading to poor quality, tough carcass. After sexual maturation, cows undergo cyclical estrus, can results in unplanned pregnancies, and affects fattening. Thus castration is an effective way to control sexual desire and reproductive capacity of livestock that reduces aggressive and sexual behaviors. It also improves feed conversion, meat, and carcass quality. The meat of sexually mature male pigs often emits an unpleasant odor commonly known as “boar taint” mainly caused by androstenone that has a serious impact on pork quality.



Immunization with GnRH in post-pubertal male, testicular size is reduced, while prepubertal animals exhibit arrested and/or reduced growth rates. In female, follicular development is arrested or reduced and ovaries and uteri are reduced in size.



GnRH vaccines and various animal species (wild, zoo, farm, and domestic) in which they have been tested.



PZP vaccines various animal species (wild, zoo, farm, and domestic) in which they have been investigated.

(Naz and Saver, 2016)



➤ **(Zona Pellucida Of Porcine) PZP Vaccine**

ZP is an **extracellular glycoprotein layer** surrounding mammalian oocyte and is involved in **species-specific sperm-egg binding**, initiation of acrosome reaction, and protection of oocyte before implantation. The zona pellucida of the oocyte consists of **three glycoproteins**. These glycoproteins have been named zona proteins 1, 2 and 3 (ZP1, ZP2 and ZP3). **Zona proteins 1 and 2 are structural proteins** providing the **structural integrity** of the zona. **Zona protein 3** is much like a **receptor** binds to proteins on the spermatozoal membrane.

**Can PZP be used in vaccine formulation meant for other mammalian species?**

Although the amount of individual ZP components differ, it is conserved among mammalian species; thus, PZP can be used in other species. Porcine ZP3 has -72.8% amino acid (aa) sequence homology with cat ZP3, 75.7% with dog, 83.6% with cow and 65.6% with mouse (Harris *et al.*, 1994). PZP antibodies **do not bind to any other cell/tissue besides ZP**, thus is devoid of unwanted interactions. However, in some large carnivores, it showed limited to no contraceptive effect along with high incidence of injection site reaction and abnormal aggressive behavior.

➤ **An ideal Contraceptive vaccine (CV) should provide –**

A long-term effect without any health hazards, should be reversible, except in situations where permanent sterility is desirable, reduction of **sexual/ aggressive behavior** is advantageous in zoo, farm, and domestic but **not wild animals** where it is needed to maintain herd hierarchy. For wild and zoo animals, remote delivery is important to avoid stress of capturing and restraining animals for hand injection. CVs for use in wild and farm animals should not pass through the food chain. A CV must be cost-effective compared to other fertility control methods. Fertility control of various (wild/zoo/farm/domestic) animal populations gained impetus in the 1960s, and several modalities available for human use such as steroidal pills, implants, and barrier methods were employed in animals. However, these contraceptive modalities have several limitations including daily intake requiring multiple doses for efficacy.

**Advantages of Immunological Approaches**

Increases litter size in sheep and twinning rates in cattle (**Prolificacy**). Improves conditions like endometritis, infertility, abortion. They require **only periodic intake**. They are free from **user failure risk**. They are **physiological** in the sense that immunological methods mobilize the body's





own machinery without the requirement of constant medication with synthetic compounds. Immunization will no doubt require injection of the antigen, but the **amount administered is small** (microgram or milligram quantities of proteins), and that too at periodic intervals with reversible response. An **alternative to surgical sterilization** for fertility control of animals (wild, zoo, farm, and domestic) is needed to prevent **problems related to overpopulation**, including culling and relocation, domestication. The **increase of lifespan and body condition scores** was attributed to a reduction in energy consumption due to lack of pregnancy and lactation.

### Disadvantages

A reduction of sexual/ aggressive behavior can harm wild life herd hierarchy. For wild and zoo animals, remote delivery is needed to avoid stress of capturing and restraining animals for hand injection. There is high incidence of injection site reaction and abnormal aggressive behavior. Due to the pelletized nature of the vaccine components, it cannot be delivered remotely. High costs are a major hindrance in large-scale population management.

### Conclusion

Immunological approaches are effective to increase the fertility and conception rates as well as to reduce fertility. But still some more research and improvement is needed to make it easily available.

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