



# Reproductive Immunization of Domestic and Wild Animals: Review

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**Abstract** – This review was carried out to provide a piece of information about reproductive immunization on domestic and wild animals. The immune system of animals plays an important role in reproduction. For inducing or controlling fertility according to human's desire, interventions of immunization are an important aspect of management in domestic and wild animals. Immunization against reproductive hormones such as gonadotrophic hormones, gonadal steroids, oxytocin, prostaglandin F<sub>2</sub>, inhibin, luteinising hormone releasing hormone, luteinising hormone, follicle stimulating hormone has provided a significant effect on reproduction of animals. Immunisation against gamete antigens such as against sperm antigens and zona pellucida antigens has shown also a profound effect on reproduction of domestic animals (cattle, sheep, goats, poultry, pigs, horses) and on wild lives. Therefore carrying out different types of reproductive immunization based on their scientific perspectives and procedures with a great precaution helps to induce or suppress of fertility in animal reproduction.

**Keywords** – Immunization, Fertility, Reproductive Hormones, Reproductive Gametes.

immunogen in a controlled way, its body can learn to protect itself: this is called active immunization. The most important elements of the immune system that are improved by immunization are the T- cells, B- cells, and the antibodies B- cells produce. Memory B- cells and memory T- cells are responsible for a swift response to a second encounter with a foreign molecule. Passive immunization is when these elements are introduced directly into the body, instead of when the body itself has to make these elements [6]. Active immunization was considered likely to be more practical than a passive system if a reproducible and controlled response could be achieved [7]. Different authors studied the effect of reproductive immunizations on mouse, rats, pigs, elks, elephants, rabbits, monkeys, dogs, cattle, sheep, goats, poultry, equines and human in different times about fertility induction and fertility control. Therefore to know and understand more about the reproductive immunization of domestic and wild animals within its applications and impacts this review is made from different sources.

## I. INTRODUCTION

Reproduction is an essential function of every animal species, and its realization depends on a complex of interrelated neural, endocrine, immune, and behavioral reactions [1]. The growth and maturation of ovarian follicles is a fundamental process for effective reproduction in farm animals. However, reproduction is one of the most serious biological problems that markedly influence the efficiency of livestock production. In farm animal species fecundity augmentation, control of fertility and sexual and aggressive behaviour are important factors of animal productivity [2]. Immunology is a powerful tool for studying both normal fertility and infertility of farm animals [3]. Therefore immunological techniques have been developed to monitor reproduction under practical conditions. One of the recent mechanisms to monitor reproduction is reproductive immunization. Immunization is the process by which an individual's immune system becomes fortified against an agent [4]. Immunological intervention is directed depends on whether the induction of fertility or maintenance of sexual behaviour and secondary sex characteristics are important considerations [5]. This system is exposed to molecules that are foreign to the body, called non-self, it will orchestrate an immune response, and it will also develop the ability to quickly respond to a subsequent encounter because of immunological memory. This is a function of the adaptive immune system. Therefore, by exposing an animal to an

## II. LITERATURE REVIEW

### 2.1. Immunisation against Reproductive Hormones

According to the report of [5], the endocrine control of reproduction in mammals involves hormone signalling between the brain, pituitary gland and gonads. Luteinising hormone releasing hormone (LHRH) is released from the basal hypothalamus-median eminence and stimulates the anterior pituitary gland to secrete luteinising hormone (LH) and follicle stimulating hormone (FSH), which together orchestrate testicular and ovarian function. The gonads in turn secrete a range of protein and steroid hormones. Gonadal steroids serve diverse functions which include the regulation of LHRH, LH and FSH secretion through feedback mechanisms, stimulation of brain centres associated with sexual and aggressive behaviour, and maintenance of accessory sex tissues and secondary sex characteristics. Since hormones are transported in the general circulation they are readily accessible to antibodies. It has therefore been argued that antibodies specific for a particular hormone will bind that hormone in the circulation and consequently block its biological actions. This concept has been largely substantiated for reproductive hormones. It should be noted that gonadal steroids and LHRH are not immunogenic and therefore require conjugation to antigenic carrier proteins for immunisation [8]. Gonadotrophic hormones are immunogenic when used inter specifically and do not necessarily require conjugation. However, there appear to



be some improvements in antibody responses when gonadotrophic hormones are conjugated to carrier proteins [9].

### 2.1.1 Immunization against Luteinising Hormone Releasing Hormone (LHRH)

LHRH can be considered as the trigger for the reproductive hormone cascade. As such, immunological neutralisation of LHRH would be expected to block pituitary secretion of LH and FSH and result in gonadal quiescence. Animals immunised against LHRH before puberty would remain prepubertal, whilst sexually mature animals would essentially revert back to a prepubertal condition. Initial studies on LHRH immunisation in laboratory animals confirmed that reproductive function was suppressed [10]. Similar findings were subsequently reported for diverse mammalian species. Immunisation against LHRH is of particular interest in farm animals where control of sexual and aggressive behaviour, as well as fertility, is important. Cattle have been the focus of recent studies because of the potential practical applications of LHRH immunisation as an alternative to surgical and chemical sterilisation under different management systems. The efficacy of LHRH immunisation in suppressing fertility in female cattle was demonstrated in principle both under feedlot [11] and range [12] conditions.

In a recent study the potential of LHRH immunisation to delay puberty was examined in heifers in a tropical environment. Groups of heifers were immunised either at 6 and 10 months of age or at 6, 10 and 16 months. Heifers immunised at 6 and 10 months attained puberty at the same time as contemporary controls and showed normal conception rates. In contrast, heifers immunised at 6, 10 and 16 months showed a delay of puberty. The latter heifers did eventually attain puberty and conceived [13]. In both groups of heifers, circulating LH concentrations were suppressed after secondary immunisation at 10 months of age. Therefore, in heifers immunised at 6 and 10 months, the reproductive axis was suppressed for a time after immunisation, but returned to apparently normal activity in sufficient time for normal transition into puberty. This was associated with declining anti-LHRH antibody titres [13]. A tertiary immunisation at 16 months maintained circulating antibody titres and delayed the onset of puberty. Young bulls immunised against LHRH also show a delay in the onset of puberty and sexually mature bulls undergo atrophy of the testes and a decline in circulating testosterone to castrate levels.

### 2.1.2 Immunisation against Gonadotrophic Hormones

The gonadotrophins LH, FSH and human chorionic gonadotrophic (hCG) share a common subunit but differ in the subunit. Immunisations can be carried out using the purified native hormones comprising the  $\alpha$  and  $\beta$  subunits, but it is generally thought that antibodies of increased specificity are obtained if only the  $\beta$  subunits of the respective hormones are used [14].

Selective immunisation against either LH or FSH has been used to study specific hormonal requirements for gametogenesis and steroidogenesis [15]. It has also been

considered that immunisation against FSH in males may disrupt spermatogenesis without influencing the normal production of steroid hormones. This could provide a contraceptive vaccine in species where the maintenance of sexual function is important. FSH initiates spermatogenesis during pubertal development, but may not be required to maintain spermatogenesis after puberty [16]. There is some doubt, therefore, as to whether immunisation against FSH after puberty would be effective in all species. However, it is possible that strategic FSH immunisation before puberty may induce a permanent lesion in the spermatogenic cycle regardless of species. In females, FSH stimulates early development of follicles and is therefore required on a continuing basis. Accordingly, FSH immunisation may have potential as a contraceptive vaccine in females [17]. However, females immunised against FSH may not maintain sexual characteristics because of a lack of sufficient oestrogen normally secreted by developing follicles.

Studies on LH immunisation have tended to focus on females because of the requirement for LH in follicle maturation and ovulation. In a series of studies in cycling cows [18] and young heifers [19] were able to demonstrate a consistent suppression of ovarian activity following immunisation against a bovine LH-ovalbumin conjugate. In one study, puberty was delayed for up to 8 months in heifers [20]. However, this response was achieved using an intensive immunisation schedule and it would be of interest to determine whether similar results are also obtained with a more practical immunisation protocol. Ewes immunised against bovine LH were also found to become anovular [21]. Young bulls immunised against LH showed reduced testis growth and had serum testosterone concentrations similar to surgical castrates [22]. Immunisation of heifers [19] and ewes [21] using a bovine LH-ovalbumin conjugate induces an anovulatory condition. Immunisation of young heifers using an hCG-ovalbumin conjugate induced an anovulatory condition most likely because of cross-reactivity of the antibodies with endogenous LH [19]. Active immunisation of cows against pregnant mare serum gonadotrophin induced an anovular condition which was, however, associated with cystic follicles [23].

### 2.1.3 Immunisation against Gonadal Steroids

Gonadal steroids serve important roles in sexual behaviour, accessory sex gland function and in the establishment and maintenance of pregnancy. It is therefore not surprising that numerous studies have investigated the reproductive consequences of immunisation against androgens, oestrogens and progestagens. Immunisation against testosterone in males is thought to reduce feedback on gonadotropin secretion and has been shown to promote increased testis size and increased testosterone secretion in rams [24] and bulls [13]. Although testosterone-immunised males have higher circulating testosterone levels, it was presumed that anti-testosterone antibodies would neutralise the actions of testosterone on sexual behaviour and somatic tissues. Immunisation of rams and bulls against testosterone appears not to influence sexual behaviour [24] or testicular



and accessory sex gland function[13]). Therefore, testosterone immunisation may be associated with pro-fertility rather than anti-fertility responses. Testosterone immunisation in ewes[25] and heifers [26] can stimulate ovarian activity, but this is usually excessive and causes abnormal ovarian cycles, cystic follicles and a reduced incidence of oestrus.

A second androgen that has received attention in females is androstenedione. Immunisation against this steroid increases ovulation rates and fecundity in sheep and possibly also cattle[27] However, in a study in cows, androstenedione immunisation induced aberrations in ovarian function including varying degrees of superovulation and follicular cysts [28]. It is likely that the ovulations were associated with reduced fertility.

The apparent divergent responses of females, and in particular of female cattle, to androgen immunisation are possibly related to antibody titres [29] . It appears that relatively high antibody titres are associated with anovulation, follicular cysts and reduced incidence of oestrus. Immunisation protocols which induce infertility by causing abnormal ovarian function would be acceptable as contraceptive vaccines. Oestradiol- 17 stimulates oestrous behaviour in females and also triggers the preovulatory LH surge. It could therefore be regarded as a good target for an anti-fertility vaccine. Ewes immunised against oestradiol-17 were reported to become anovular and anoestrus[30]. Unfortunately, immunisation against oestradiol- 17 can also precipitate abnormal ovarian activity including cystic follicles [31]. Oestradiol- 17 , like androgens, is therefore an unlikely candidate for a contraceptive vaccine.

Progesterone plays a critical role in the establishment and maintenance of pregnancy and is perhaps the most likely gonadal steroid to be targeted by a contraceptive vaccine in females. Immunisation against progesterone should block the actions of this steroid on the uterus and prevent implantation. It could also influence embryonic development directly. In species where progesterone priming is important for sexual behaviour, the expression of oestrus should also be influenced [32].

After active progesterone immunisation fertility was reduced in ewes [33] and their response were varied from abnormal ovarian function and anoestrus [32], but no effects on either oestrous cycle length or conception rate [34] . The reason for the lack of response may have been related to antibody titres. Plasma progesterone concentrations were ten times higher in immunised ewes, and although the percentage of free progesterone decreased, absolute amounts of free progesterone were similar for immunised and control ewes [34]. Progesterone immunisation in ewes causes changes in ovarian function which include increased ovulation, ovulation in the presence of corpora lutea and variable cycle lengths [33,32]. These changes in ovarian function are unlike the undesirable responses observed following immunisation against androgens and oestrogens, in particular the occurrence of cystic follicles. Immunisation against progesterone might therefore be acceptable as a

contraceptive vaccine for livestock provided that oestrous behaviour and conception are also suppressed.

#### 2.1.4 Immunisation against Oxytocin

Oxytocin secretion from the ovary commences with preovulatory follicles and proceeds throughout the luteal phase [35]. It has been suggested that oxytocin secreted in the late luteal phase acts on the uterus to stimulate prostaglandin F<sub>2</sub> (PGF<sub>2</sub>,) production, which in turn initiates luteolysis [36] . In support of this concept, immunisation of ewes and goats [37] against oxytocin extended the oestrous cycle. More recently, it was found that ewes actively immunised against oxytocin showed significantly reduced conception rates[38].

#### 2.1.5 Immunisation against Prostaglandin F<sub>2</sub>

PGF<sub>2</sub> is secreted by the uterus and induces lysis of the corpus luteum. Accordingly, neutralisation of endogenous PGF<sub>2</sub> should result in maintenance of the corpus luteum. This was demonstrated in heifers [39] where active immunisation against PGF<sub>2</sub>, extended oestrous cycle lengths and conception was blocked for up to 4 months in some animals[39]. Immunisation against PGF<sub>2</sub>, is of particular interest since continued secretion of progesterone may provide an endogenous anabolic effect in meat producing heifers. However, pregnant animals should not be immunised against PGF<sub>2</sub>, since this would interfere with parturition.

#### 2.1.6 Immunisation against Inhibin

Inhibin is secreted by the gonads of mammals and it plays an important role in the control of FSH release [40]. [2] reported that, Inhibin is a non-steroidal glycoprotein hormone of gonadal origin with major action as negative feedback control of the production of Follicle Stimulating Hormone (FSH) by anterior pituitary gland which in turn modulates male and female reproductive functions. Its physiological role has led to the development of inhibin based immunogens for fertility enhancement in farm animals. It is envisaged that a reduction of endogenous inhibin secretion would increase FSH concentrations and thus offers a potential for increasing the number of ovulatory follicles in the ovary. Immunization against inhibin has been reported to be a useful method for inducing multiple ovulations in farm animals. Inhibins play important roles in the regulation of fertility based on their dual inhibitory action on the process of folliculogenesis in the ovary and FSH secretion by the pituitary. Inhibins are also recognized as paracrine ovarian and testicular regulators and have multiple paracrine effects in the utero-placental unit, representing a promising marker for male and female infertility, gynecological and gestational diseases. Inhibin essentially suppresses FSH, immunisation against inhibin is being examined as a way of increasing ovulation rates in females [42] and generally increasing fertility in males [43] . However, in a study in ewes, active immunisation against the 43 subunit of inhibin impaired conception rates [44]. It was suggested that differential processing of the subunit of inhibin, either before or after dimerisation with the sub unit can have either a positive or negative influence on fertility.



## 2.2 Immunisation against Gamete Antigens

The interaction between eggs and sperm at fertilisation involves specific surface antigens present on gametes of both sexes [45]. These antigens are presently the focus of intense study owing to their potential as targets for contraceptive vaccines. The particular attraction of gamete antigens for immunological contraception is that other reproductive functions should remain normal. Immunising against surface antigens of gametes is that: in immunised males; antibodies bind to sperm both during maturation or ejaculation and, in immunised females; antibodies bind to ova during development, or are released into reproductive tract fluids and bind either ova or sperm [46]. Antibody binding to sperm or ova will block gamete interactions required for fertilisation. A precedent for gamete antigen vaccines is provided by the infertility associated with autoimmunity to sperm antigens in both males and females.

### 2.2.1 Immunisation against Sperm Antigens

The best characterised of the sperm antigens are FA-1 [47], PH-20 [48], SP-10 [50], lactate dehydrogenase-C4 [51], Fertility antigen-1 (FA-1) and germ cell antigen (GA-1) [47].

The sperm surface antigen, PH-20, was originally isolated from guinea pig sperm and shown to be essential for fertilisation in this species [52]. In one study, active immunisation against PH-20 induced total infertility in 6/6 male and 25/25 female rabbits [52]. The gene for PH-20 was recently cloned and shown to be present in rabbits and bulls including man [48]. This wide distribution suggested that PH-20 has been conserved during evolution and it might therefore be a particularly important antigen in egg-sperm interactions. However, to date, expression of the gene has only been demonstrated in guinea pigs. It was suggested that species specificity could be achieved through subtle changes in the binding domains of PH-20 [48]. The sperm antigen, SP-10, has received similar attention as FA-1 as the basis for a contraceptive vaccine in man [53]. SP-10 is present in the acrosome and on the surface of sperm following the acrosome reaction [53]. It is therefore considered to be important in fertilisation. A further antigen of interest in male gamete contraception is the sperm specific isozyme lactate dehydrogenase-C4 [51]. Reduced fertility after immunisation of females with LDH-C4 was observed in rabbits [54]. However, in a recent study immunisation of male mice against LDHC4 reduced fertilisation rates [55].

### 2.2.2 Immunisation against Zona Pellucida Antigens

The zona pellucida (ZP) forms a coating around mammalian eggs and is comprised primarily of three glycoproteins, ZP1, ZP2 and ZP3 [56]. The ZP glycoproteins form a coating around the ovum and are involved in sperm binding during penetration of the zona. The glycoprotein ZP3 was identified as the major sperm receptor and targeted as the most likely female gamete antigen for a contraceptive vaccine [57]. Immunisation of females against whole ZP and ZP antigens has shown to reduce fertility in a number of species. In an interesting practical demonstration of ZP immunisation, feral mares were remotely immunised with porcine ZP using dart

guns [58]. This was shown a successful result in preventing pregnancies in the majority of immunised mares.

Immunisation against ZP antigens was associated in some studies with abnormal ovarian function and altered reproductive hormone profiles [59]. This raised concerns about the use of ZP antigens for contraceptive vaccines in some species, particularly man. However, it appears that ovarian function returns to normal over time and normal fertility should also be restored [60]. It was suggested that recombinant procedures could be used to produce ZP peptides that do not induce undesirable responses at the ovaries [61].

### 2.3 Immunisation against the Conceptus

The mammalian conceptus undergoes development in the face of a functional immune system. Early events of pregnancy involve complex interactions between maternal tissues and the developing trophoblast. These interactions include immune mechanisms which are poorly understood [62]. The trophoblast itself comprises different cell types that express different antigens which potentially could be targeted by antibodies [63]. According to [64], Conceptus (embryo/fetus and associated extra embryonic membranes) growth and development is dependent initially on the endometrium and then on both the endometrium and placenta once implantation and then placentation are completed by midpregnancy. Histotrophic nutrition is primarily derived from the secretions of uterine glands that bathe the conceptus and are absorbed by placental areolae. Areolae are unique placental structures in ruminants and pigs that develop over the mouth of each uterine gland as specialized areas for absorption and transport of uterine histotroph. Hematotrophic nutrition is derived from maternal blood and is, in large part, influenced by uterine blood flow. Following placentation, the requirement for histotroph remains critical in domestic animals with an epitheliochorial (pig) or synepitheliochorial (sheep, cattle, and goat) placenta. Histotrophic and hematotrophic nutrition influence conceptus development, onset of pregnancy recognition signals, and fetal-placental growth in the ungulate uterus. It is becoming clear that placental hormones act in a paracrine manner on the uterus to maximize production of histotroph. Knowledge of basic mechanisms regulating uterine development and function in the neonate and adult is necessary to develop biotechnological tools to increase uterine capacity and fetal growth, thereby enhancing reproductive efficiency and profitability of animal production agriculture.

## 2.4 Effects of Immunization on Domestic Animals

### 2.4.1 Immunization on Cattle

#### 2.4.1.1 Cow

[65] reported that, mature beef cows were actively immunized pre partum (5 cows) or postpartum (10 cows) against a PGF-2 ovalbumin conjugate or against ovalbumin alone (control; 5 cows). All cows in the control group exhibited first oestrous cycles which were of short duration (12 days). In the pre-partum PGF-2 immunized cows, lifespan and progesterone secretion of the first corpus luteum formed post-partum was maintained for > 39 days. Specific serum binding to [3H] PGF-2 in pre-

partum and post-partum PGF-2 immunized cows was elevated. Lifespan of the first corpus luteum formed in post-partum PGF-2 immunized cows was short (<10 days; 1 cow), normal (mean = 22 days; 4 cows) or maintained (>31 days; 5 cows). Luteal lifespan was dependent upon serum PGF-2 antibody titres, with cows exhibiting higher titres frequently having prolonged luteal lifespans after first ovulation. According to [65] active immunization of beef cows against PGF-2 extends the lifespan and progesterone secretion of corpora lutea anticipated to be short-lived. These results support the concept that the shorter lifespan of some corpora lutea in post-partum cows is due to a premature release of PGF-2 from the uterus.

According to the report of [66], Holstein cows were administered zona pellucida (ZP) DNA vaccine and used to determine the potential of recombinant rabbit ZP glycoproteins (rZP) as immune contraceptive antigens. Four of the six cows in ZP treatment groups developed antibody titer levels with similar linear responses over time. These cows also experienced reduced ovarian function as indicated by decreases in follicular and luteal activity. Estrous activity was observed in all cows and decreased in ZP treatment cows in comparison to controls.

#### 2.4.1.2 Heifer

The study was conducted by [67], to evaluate the effect of conjugation of GnRH to human serum albumin (HSA; HSA-GnRH), ovalbumin (OA; OA-GnRH), or keyhole limpet hemocyanin (KL; KL-GnRH) and use of Freund's complete adjuvant (FCA), Freund's incomplete adjuvant (FIA), or DEAE dextran (DD) and mineral oil (MO) on antibody titers to GnRH, luteal activity, and pregnancy rate of beef heifers. Their result has shown that Titers against GnRH for heifers immunized against HSA-GnRH with FCA, DD+MO, or DD+FIA were greater than titers for HAS GnRH with FIA or control heifers ( $P < 0.01$ ). Body weight was reduced ( $P < 0.05$ ) in control and FCA heifers compared with FIA, DD+MO, and DD+FIA heifers. Heifers immunized with DD+MO and DD+FIA had fewer granulomas in mammary glands than heifers treated with FCA ( $P < 0.01$ ). Immunization of heifers against OA-GnRH, KL-GnRH, or HSA-GnRH suppressed luteal activity ( $P < 0.01$ ) for 23, 16, and 12 week, respectively, and antibody titers against GnRH were greater ( $P < 0.01$ ) for 19, 5, and 7 week, respectively, compared with heifers immunized against the carrier proteins. At 8 week after primary immunization, heifers were exposed to fertile bulls for 24 week. Pregnancy rate was less ( $P < 0.01$ ) for 3-booster heifers (13%) compared with control (83%) and 2-booster (62%) heifers. The same author revealed that, the immunization against GnRH, conjugated to OA and emulsified in DD+FIA, does not influence ADG and produces sufficient titers against GnRH to prevent estrous cycles with few mammary granulomas. Immunization against GnRH with 3 booster immunizations prevented luteal activity and pregnancy in most beef heifers for more than 4 months.

Another study was conducted by [68], to determine the effects of active immunization against prostaglandin F<sub>2</sub> (PGF) on estrous activity and performance traits of beef

heifers: 1) heifers (controls) given 3.3 mg of human serum albumin (HSA) on d 0 (primary) and 27 (booster), and 2) heifers (PGF-immunized) given 3.3 mg of PGF-HSA on d 0 and 27. The adjuvant was DEAE dextran, Plasma progesterone concentrations were used to monitor corpus luteum (CL) presence; PGF antibody titers were determined every 2 wk. Antibody titers for PGF immunized heifers increased to a peak (43.2.9% binding at a plasma dilution of 1:1,250) on day 55  $\pm$  4.6. Antibody titers were greater ( $P = 0.02$ ) in PGF immunized than in control heifers by day 15 and remained elevated ( $P = 0.001$ ) throughout the experiment. 96% of PGF-immunized heifers formed persistent CL with a mean duration of 129  $\pm$  6.4 days. The mean number of estrous periods per heifer were less for PGF-immunized (1.5  $\pm$  .27) than for control heifers (7.0  $\pm$  .32). Mean daily live weight gain of the PGF-immunized heifers was decreased ( $P < .05$ ; .75  $\pm$  .024 kg) compared with that of controls (.83  $\pm$  .014 kg), largely due to a 31.5% decrease during the 28-days period after booster. The authors concluded that, PGF immunization suppressed the estrous behaviour of 96% heifers; however, there was a significant reduction in live weight gain for the 28 days period after booster.

According to [26] Yearling heifers were actively immunized against 8 mg testosterone-3-carboxymethyl-oxime-ovalbumin in Non-Ulcerative Freund's Adjuvant, with or without the addition of Corynebacterium parvum (Groups A and B, respectively; N = 4 for each group). After the priming injection, Groups A and B were boosted twice at 4-monthly intervals. Control heifers (N = 9) were not injected. All treated animals except one gave a measurable antibody response, and all responding animals became anoestrous and displayed ovarian cysts after the first booster injection. There were no apparent differences between treatments, and so results for Groups A and B were pooled. At 25 weeks after the second booster 3 of the 7 responding, anoestrous heifers resumed cyclicity; one with two consecutive double ovulations, and one with one double ovulation. The 3rd heifer showed 4 corpora lutea, then became anoestrus again. The 4 remaining acyclic heifers, and the control heifers, were intensively blood sampled; the anoestrous heifers showed significantly higher mean LH and significantly lower mean FSH concentrations and higher LH pulse frequency than did the control animals. These heifers remained anoestrous for 11 months after the second booster, at which point they were injected with GnRH and PGF-2 alpha; only 1 heifer resumed ovarian cyclicity. These results indicate that it is possible to increase ovarian activity in cattle by active immunization against testosterone, but that there is a high incidence of anoestrus.

#### 2.4.1.3 Bull

[69] conducted their study to evaluate the ability of trenbolone acetate (TBA) administered in tandem with LHRH immunization to suppress reproductive function in bulls and examine the effects of LHRH and androgen (TBA) signalling on pituitary gland function. The result showed that LHRH immunization decreased synthesis and storage of LH and decreased storage, but not synthesis of



FSH in bulls. The increased synthesis of LH and FSH in nonimmunized, but not LHRH-immunized steers suggests that castration removes the negative feedback on gonadotropin synthesis but that LHRH is still needed for release of these hormones. Androgen replacement with TBA did not restore the negative feedback control of gonadotropin synthesis. [70] used feedlot steers to show that a single immunization with GnRH conjugated to KLH was sufficient to raise anti-GnRH titers and reduce LH secretion for more than 28 weeks. Similar immunization of bulls retarded testis growth but did not affect performance characteristics, perhaps due to the late age at immunization 10.5–11 months. In a subsequent study [71], immunization of bulls at 3.6 months of age did improve feedlot gain, carcass weight, and loin eye area relative to steers. [72] reported that immunization of crossbred bulls against GnRH did not affect feed intake, but did alter carcass fat and protein content; bulls with the highest anti-GnRH responses tended to be similar in composition to steers, whereas those with lesser responses were closer to the control bulls. In a study comparing ages at immunization, [71], found that immunization at 7 months of age was most effective in reducing testicular size at slaughter at 18 months of age, although most carcass characteristics were unaffected. In a similar age study, [73], reported that immunization of Friesian bulls at 2.5, 4, or 7.5 months of age resulted in a delay in development of sexual and social behaviors, and that there was no practical reason to immunize before 7.5 months.

The study was conducted by [74] to evaluate the effect of immunization against gonadotropin-releasing factor (GnRF) with Bopriva<sup>®</sup> in prepubertal bull calves. The results showed that compared to control animals, inhibition ( $P < 0.05$ ) of the prepubertal LH secretion was observed in vaccinated calves at weeks 10 and 12–14 after the first vaccination. In vaccinated calves testosterone concentrations decreased after the booster injection to values below 0.5 ng/mL serum and remained for at least 22 weeks at this low level. Animals vaccinated with Bopriva<sup>®</sup> showed a delay in testes growth and smaller scrotal circumference. Puberty occurred at the age between 46 and 55 weeks in vaccinated and between 38 and 52 weeks in control animals and body weight gain was similar in both groups. All vaccinated bulls attained spermatogenic capacity at slaughter when they were 68 weeks old.

#### 2.4.2 Immunization on Sheep

##### 2.4.2.1 Ewes

According to the report of [75], Two adjuvants, Freund's complete adjuvant (FCA) and GNE (proprietary product; Intervet Ltd, The Netherlands), were used to immunize cyclic Finnish Landrace ewes against a prostaglandin F-2 alpha-human serum albumin (PGF-HSA) conjugate. A persistent CL formed, on average, 10.0, 10.0, 29.8 and 32.5 days after primary immunization (pooled s.e.d. = 14.6) in 6/6 FCA-5 mg, 6/6 FCA-15 mg, 5/6 GNE-5 mg and 4/4 GNE-15 mg-assigned ewes, respectively; these CL were maintained for, on average, 138.7, 139.0, 127.8 and 129.0 days, respectively. As the report of [76], Partial neutralization of bone morphogenetic protein 15 (BMP15)

bioactivity by immunization is known to increase ovulation rate in sheep. They tried to compare the efficacy of a BMP15 vaccination treatment on lamb production to that of commercially-available androstenedione-based vaccines that are used for this purpose. The author showed that immunization against either androstenedione or BMP15 increased ovulation rate. Androstenedione vaccination also increased the number of lambs weaned ( $P < 0.05$ ). Bone morphogenetic protein 15 vaccination altered the pattern of the number of lambs weaned, but no increase in lamb production was observed as more ewes produced zero or three lambs. Overall, androstenedione or BMP15 vaccination did not significantly affect embryo or fetal survival or lamb performance independently of the effects of these treatments on ovulation rate.

The study was conducted by [77] to investigate the role of inhibin in the distribution of healthy and atretic antral follicles and the secretion patterns of gonadotrophins. Ewes were actively immunized against either N or C of the inhibin a subunit with a primary injection and three booster injections. The control ewes received adjuvant only. The ovaries were removed either before or at 24 h after hCG administration in a synchronized follicular phase 48 h after removal of intravaginal progesterone pessaries. The authors revealed that the mean number of corpora lutea observed per ewe with corpora lutea was not significantly different in ewes immunized against N (2.4; N-immunized ewes) or C (2.6; C-immunized ewes), and control (2.4) ewes, although some corpora lutea appeared cystic in the immunized ovaries. Compared with luteal phase concentrations, mean basal FSH concentrations in the early follicular phase were significantly increased in the C-immunized ewes, similar in N-immunized ewes and reduced in control ewes. No differences were observed in any of the LH parameters. Before hCG treatment, healthy antral follicles > 1 mm in diameter were not observed in any of the 52 follicles in the C immunized ewes and were observed in one of 37 follicles from N-immunized ewes compared with 19 of 28 follicles in control ewes ( $P < 0.0001$ ). For healthy antral follicles < 1 mm in diameter, there were 72 of 85 follicles in the C-immunized ewes, 79 of 81 follicles in the N immunized ewes and 81 of 82 follicles in the control ewes. According to the report of [77] immunization against N and C may result in disruption of the normal processes of antral follicular growth and maturation independent of the concentrations of FSH and LH.

[78] studied to evaluate the immunization of sheep against GnRH early in life: effects on gonadotropins, follicular growth and responsiveness of granulosa cells to FSH and IGF-I in two breeds of sheep with different prolificacy (Romanov and Ile-de-France): The prolific Romanov (R, ovulation rate = 3) and non-prolific Ile-de-France (IF, ovulation rate = 1) breeds were compared for their ovarian sensitivity to gonadotropins and IGF-I before puberty. For this purpose, the effects of in vivo immunization against GnRH on populations of ovarian follicles and in vitro sensitivity of granulosa cells to FSH and IGF-I were studied in prepubertal lambs from both



breeds. Seventeen prepuberal lambs of each breed were actively immunized against GnRH between 3 weeks and 6 months of age. Relative to untreated lambs, FSH levels at 4, 5, and 6 months of age were (respectively) 41%, 25% and 29% for IF, and 43%, 24%, and 36% for R lambs. In a first experiment, histological analysis of ovaries was performed. Immunization treatment decreased the number of small (100-390 microns in diameter) and large size follicles (< 1500 microns) in both breeds at 6 months of age. In both breeds, gonadotropin (FSH-LH-hCG) treatment increased the number of large size follicles (< 1500 microns in diameter) and induced the formation of preovulatory follicles in immunized as well as untreated lambs. The ovulation rate was less in immunized animals, but it was not different between breeds. In a second experiment, the effects of FSH and IGF-I were studied on granulosa cells from follicles between 1000 and 2000 microns in diameter. In both breeds, IGF-I increased granulosa cell proliferation, but enhanced progesterone secretion was observed only in R lambs after FSH and IGF-I stimulation. Granulosa cell response to FSH treatment was lost by immunization, whereas response to IGF-I remained unchanged in both breeds. These results indicate that long-term immunization of prepuberal lambs against GnRH reduced systemic concentrations of FSH, follicular development, and response to gonadotropins *in vivo*, similarly in the prolific R and the non-prolific IF breed. However, granulosa cells from R lambs had higher steroidogenic capacities and were more responsive to FSH. Therefore the result suggests that IGF-I could play an important role in regulating growth of small follicles both in immunized and non-immunized lambs.

#### 2.4.2.2 Ram lambs

[79] immunized ram lambs against GnRH at 16–20 weeks of age and gave secondary injections 6, 12, and 28 weeks later. Although no slaughter data were presented, immunized rams had essentially no LH or testosterone response when challenged with a GnRH analog at 27 weeks, and had no increase in LH concentrations in response to castration at 90 weeks; they also had an 81% reduction in testis size relative to control rams. Also, [[80] reported that active immunization against GnRH in crossbred lambs produced carcasses that were similar to castrate animals, except that backfat thickness was not different from intact control lambs.

[81] immunized Merino ram lambs either before puberty or around the time of puberty and followed them through 2 years of age secondary injections were administered 10 weeks after the first immunization in both groups. Growth rates of immunized and control rams did not differ throughout the study, although prepubertal immunization did delay testicular growth until about 27 weeks of age. Most of the rams had recovery of testicular function once anti-GnRH titers dropped, but a few immunized rams still had subnormal sized testes at 115 weeks of age. [82] used GnRH immunization to show that continuous LH infusion was equivalent to high frequency, low amplitude replacement of LH in restoring normal Leydig cell function in yearling rams. And lastly, [83] used GnRH immunization in conjunction with diet to show that there is

a nutritional component of normal testicular growth that is independent of GnRH.

#### 2.4.3 Immunization on Goats

##### 2.4.3.1 Female Goats

According to the study of [84] Approximately 150 days before expected breeding time, 12 female goats (3 months of age) were actively immunized against ovine leptin. Booster injections were given throughout the following year. Control animals (n = 6) were sham-immunized. After the first observed oestrus, a buck was introduced and goats were mated. The result showed that nine of the immunized goats developed titres within 3 months and had elevated serum concentrations of leptin compared with controls ( $P < 0.0001$ ). Hematological parameters and blood chemistry were not affected by the immunization. No differences were detectable in all reproductive parameters recorded. Serum insulin was higher in immunized goats during the frequent blood sampling series of day 287 after the first immunization.

[86] reported that at the beginning of the breeding season, 35 goats from local Greek breeds were immunized against androstenedione by two injections of Fecundin 3 weeks apart while 40 goats were untreated. Fertility at the first oestrus following buck introduction was unaffected by treatment while ovulation rate ( $P < 0.01$ ) and litter size ( $P < 0.001$ ) were significantly increased in immunized goats. Fetal growth was unaffected by immunization as evidenced by similar birth weights of kids in treated and control goats for a given litter size. Growth rates of kids throughout their first month of age was depressed in kids born from immunized goats ( $P = 0.05$ ). Overall milk production between 5 and 19 weeks after kidding was marginally increased ( $P = 0.08$ ) in immunized goats. Hence, immunization against androstenedione is likely to be profitable in goats.

##### 2.4.3.2 Male Goat

[87] Godfrey et al. (1996) immunized feral adult bucks in the summer with Vaxtratew and compared their reproductive characteristics, behaviour and male odor with non-immunized bucks and castrates. Secondary immunizations at 2 or 4 weeks after the first produced equivalent responses, with a 78% and 63% reduction in testicular parenchymal weight, respectively, compared with control bucks 394 days after immunization. Odor score as well as concentrations of LH, FSH, and testosterone were all reduced in immunized bucks by 56 days after the first immunization. Body weights did not differ between immunized and control bucks, although control bucks had reduced feed intake during the same period of time

#### 2.4.4 Immunization on Poultry

The role of testosterone in the ovulatory process in hens has been largely neglected. The study was conducted by [88] to evaluate if testosterone plays an important role on the ovulatory process in laying hens. The effect of active and passive immunization against testosterone on ovarian follicular development and oviposition was studied and concluded that testosterone immunization hampers egg-laying without affecting ovarian follicular development, suggesting that testosterone has an important role in the



ovulatory process in laying hens. [89] evaluated the effect of immunization of sexually immature pullets against poly-alpha-L-glutamic acid on long-term fertility. Poly-alpha-L-glutamic acid (PGA) is a polypeptide synthesized within the upper oviduct and uterovaginal junction of the hen. Sexually immature pullets were immunized against PGA. These virgin pullets, and immunized and non-immunized controls, were inseminated at 25 weeks of age and then monthly throughout a 30-week egg production interval. While birds immunized against PGA were free of PGA as evidenced by an absence of immune precipitation when rabbit anti-PGA serum was overlaid with oviduct extract, immunization had no effect on long-term fertility and therefore is unlikely to have an immunosuppressive or antigen-masking biological role with respect to spermatozoa. The authors suggested that instead that PGA could act as a calcium binding protein.

Photostimulation of retinal photoreceptors appears to inhibit reproductive activity in birds [90]. In the present study, the involvement of serotonin and vasoactive intestinal peptide was investigated in relation to reproductive failure associated with retinal photostimulation on Hens at 23 wk of age. Five hens from each room served as controls, five hens were immunized against vasoactive intestinal peptide, and five hens received parachlorophenylalanine, an inhibitor of serotonin biosynthesis. Parachlorophenylalanine treatment increased reproductive performance and mRNA expression of GnRH-I, LH-beta and FSH-beta ( $P < 0.05$ ) did not differ from those of the control group. Immunization against vasoactive intestinal peptide reduced plasma concentration and pituitary mRNA expression of prolactin but did not affect expression of gonadal axis genes. Collectively, the results suggest that retinal photostimulation inhibits the reproductive axis through serotonin and not through vasoactive intestinal peptide.

#### 2.4.5 Immunization on pig

Male odor is a particular problem in male boars after puberty, when adult levels of androgens are produced by the testes. [91] immunized crossbred boars against GnRH coupled to human serum globulin with either FCA or muramyl dipeptide as adjuvant at 12 weeks of age, followed by secondary immunizations at 16 and 18 weeks of age. Adjuvant was not a significant factor for any characteristic throughout the study. Compared to adjuvant-immunized controls, immunized boars had undetectable LH and testosterone concentrations at 22 weeks of age, a 70% reduction in testicular weight, and accessory sex gland weights not different than castrates. Average daily gains through 24 weeks of age were not affected by immunization and carcass characteristics were generally similar among groups at slaughter at 24 weeks; however, boar taint was reduced in immunized boars to levels not different from castrates. [92], reported similar results for boars immunized at 100 days of age with GnRH linked to BSA and injected in FCA; in contrast to FCA, boars immunized with alumina gel did not produce significant antibody titers.

Another study was conducted by [93] on Mechanism of Infertility in Male Guinea Pigs Immunized with Sperm PH-20'. PH-20, a testis-specific protein first expressed in haploid germ cells, is present on the posterior head plasma membrane and inner acrosomal membrane of mature guinea pig sperm. PH-20 is bifunctional, having a hyaluronidase activity that allows sperm to penetrate the cumulus layer and a separate activity required for binding of acrosome-reacted sperm to the zona pellucida. The immunization of male guinea pigs with PH-20 reproducibly results in infertility with a duration of 6-12 months or longer. The immunopathology in the reproductive tract of PH-20-immunized males to probe the mechanism(s) responsible for the induced infertility and found two separate effects. Remarkably, in almost all infertile, PH-20-immunized males, the caudae epididymides were empty (contained no sperm) or contained only abnormal sperm. The complete loss of normal sperm in the epididymis apparently results in infertility. A second effect was the induction of experimental autoimmune orchitis (EAO), representing the first report of EAO induced by a purified testis/sperm molecule of known functions. PH-20-induced EAO differed from EAO induced by crude testis antigens in two respects: 1) an absence of epididymitis with abscess and granuloma and 2) the presence of antibody on germ cells within seminiferous tubules and inside the cauda epididymidis. The former suggests that crude testis antigens other than PH-20 are responsible for epididymitis, and the latter suggests a possible role of antibody in EAO pathogenesis and infertility induction. Return to fertility, after 6-12 months, was accompanied by regression of EAO and reappearance of spermatozoa in the caudae epididymides.

#### 2.4.6 Immunization on Horse

The practical application of GnRH immunization in the horse was first reported by [94], who actively immunized a cryptorchid stallion as an alternative to surgical removal of an abdominal testis. Serum LH and testosterone concentrations remained low while anti-GnRH titers were high, but returned to normal over a 7-month period between secondary immunizations. Re-immunization resulted in rapid restoration of titers and a drop in testosterone concentrations to castrate levels. [95] used active immunization against GnRH to study the differential dependency of LH and FSH on GnRH input to the pituitary of horses. Long-term concentrations of LH in plasma of mares were totally suppressed by immunization, whereas FSH secretion was reduced by only 50%. Further study of these mares [95] showed that the testosterone-induced stimulation of FSH production in the pituitary which had been previously reported for castrate males as well as females;

[96], Immunization against GnRH reduced pituitary weight by 31%, reduced pituitary LH content by 91%, but only reduced pituitary FSH content by 55%. In fact, challenge with a GnRH analog that did not bind to the anti-GnRH antibodies prior to slaughter resulted in a similar FSH response in immunized and control ponies, even though the LH response was reduced by 90%. There



was no effect of immunization on prolactin response to thyrotropin releasing hormone injection or on pituitary prolactin content. Moreover, GnRH concentration in the median eminence, preoptic area, and body of the hypothalamus was unaffected by immunization, indicating that there was likely no short-loop feedback of GnRH on its own production and storage.

### 2.5 Effect of Immunization on Wildlife

Increasing attention is being focused on fertility control as a possible technique for controlling wildlife populations in urban and suburban areas [97]. In many of these areas, wildlife populations are becoming overabundant. However, in such settings, traditional hunting or lethal control programs may not be publicly acceptable. A demand has arisen for contraceptive management techniques. The most recent developments in wildlife management is immunocontraception [98]. Immunocontraception uses an animal's own immune response to disrupt reproductive function. Proteins of eggs, sperm, fertilized eggs, and reproductive hormones were developed and used for developing a vaccine for fertility control. The most widely tested immunocontraceptive vaccine for wild species is based on developing antibodies to the zona pellucida (ZP), which surrounds the mammalian egg cell. This vaccine has successfully caused infertility in some individual animals, but requires multiple treatments. Enhancement of immune response and efficiency of vaccine delivery will be necessary before this type of management strategy can be applied to wildlife control at the population level. Contraceptive treatment may alter the health and behavior of wildlife populations and therefore must be monitored closely [97].

The study was carried out by [99] to determine the efficacy of a single immunization with gonadotropin-releasing hormone (GnRH) vaccine to suppress reproductive function in pregnant female elk and to evaluate potential behavioral and pathological side-effects of treatment. GnRH vaccination did not affect existing pregnancy or calf survival during the year that it was applied; however, it reduced the proportion of pregnant females for three years. Male precopulatory behavior rates exhibited toward GnRH-vaccinated females tended to be greater than those directed at sham-vaccinated females during the second half of the breeding season, when GnRH vaccinates continued to be proceptive. Strong immune and inflammatory responses, including robust GnRH antibody concentrations in GnRH vaccinates, and sterile pyogranulomatous injection site abscesses in both groups, were consistent with vaccination. GnRH vaccine resulted in prolonged, though reversible impairment of fertility, and is associated with extended reproductive behaviors and partial suppression of hypothalamic-pituitary-gonadal axis function in captive female elk. [100] reported that the successful use of a gonadotropin-releasing hormone (GnRH) vaccine to suppress ovarian steroidogenic activity and to treat hemorrhage and anemia associated with reproductive tract pathology in an Asian elephant (*Elephas maximus*). The Repro-BLOC GnRH vaccine was administered subcutaneously as a series of 4 boosters of increasing dose from 3 to 30 mg of

recombinant ovalbumin-GnRH fusion protein given at variable intervals after initial vaccination with 3 mg protein. Efficacy was confirmed over a year after initial vaccination based on complete ovarian cycle suppression determined by serum progesterone analyses. Estrous cycle suppression was associated with a significant increase in GnRH antibody binding and subsequent decrease in serum luteinizing hormone and follicle-stimulating hormone concentrations. Ultrasonographic examinations of the reproductive tract documented a reduction in uterine size and vascularity after immunization. The hematocrit level normalized soon after the initial intrauterine hemorrhage, and no recurrence of anemia has been detected. No substantive adverse effects were associated with GnRH vaccination. The results indicate that GnRH vaccination in elephants shows potential for contraception and management of uterine pathology in older elephants.

As the report of [101], female rhesus monkeys were immunized with estrone-17-(O-carboxymethyl) oximebovine serum albumin. All 4 immunized monkeys developed circulating antibodies which bound radioactively labeled estrone and 17 $\beta$ -estradiol with equal affinity. All animals showed an increase in the plasma estrogen levels. The rate of disappearance of injected  $^3\text{H}$ -estradiol and its conversion to more polar metabolites was inhibited in the immunized animals. Two of the 4 animals became anovulatory as evidenced by low plasma progesterone levels over an extended period. Ovulation and corpus luteum formation was successfully induced in the 2 anovulatory animals by the administration of human chorionic gonadotropin. Therefore, the estrogen-dependent surge of luteinizing hormone necessary for the induction of ovulation does not occur in the anovulatory monkeys because the circulating antibodies bind the plasma estrogens and block their action.

### III. CONCLUSIONS

Based on the above information it is concluded that active immunization of beef cows against PGF-2 extends the lifespan and progesterone secretion of corpora lutea anticipated to be short-lived. Estrous activity was observed in all cows and decreased in ZP treatment cows in comparison to Controls. Immunization against GnRH with 3 booster immunizations prevented luteal activity and pregnancy in most beef heifers for more than 4 months. PGF immunization suppressed the estrous behaviour of 96% of the heifers; however, there was a significant reduction in live weight gain for the 28 days period after booster. There is possible to increase ovarian activity in cattle by active immunization against testosterone, but that there is a high incidence of anoestrus. Animals vaccinated with Bopriva<sup>®</sup> showed a delay in testes growth and smaller scrotal circumference. Immunization against N and C may result in disruption of the normal processes of antral follicular growth and maturation independent of the concentrations of FSH and LH in ewes. IGF-I could play an important role in regulating growth of small follicles both in immunized and non-immunized lambs. Immunization against androstenedione is likely to be profitable in goats. In poultry, retinal photostimulation



inhibits the reproductive axis through serotonin and not through vasoactive intestinal peptide. Remarkably, in almost all infertile, PH-20-immunized male pigs, the caudae epididymides were empty (contained no sperm) or contained only abnormal sperm. Immunization against GnRH reduced pituitary weight by 31%, reduced pituitary LH content by 91%, but only reduced pituitary FSH content by 55% in horses.

Immunological methods of contraception or sterilization show promise for effective and efficient fertility control of individual domestic and wild animals. However, many factors must be considered before implementing this type of control on wild and domestic animal populations. Knowledge of immune function may aid in an effective choice of antigen and delivery method. Such knowledge should be combined with an understanding of the normal behavior and the reproductive biology of an animal.

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- Articles
1. The Effect Of Feeding Different Levels Of Dried Tomato Pomace On The Performance Of Rhode Island Red (RIR) Grower Chicks, On International Journal Of Livestock Production Vol. 4(3), Pp. 35-41, March, 2013
  2. Performance Evaluation of Local Chicken at Enebsie Sar Midir Woreda, Eastern Gojjam, Ethiopia on World Research Journal of Agricultural Sciences & Technology Vol. 1, No. 1, May 2013, PP: 01 – 06 ,USA
  3. Carcass Characteristics of Rhode Island Red (RIR) Grower Chicks Feed On Different Levels of Dried Tomato Pomace (DTP) Published on International Journal of Advanced Research (2013), INDIA

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