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REVIEWS

New trends in immunocastration and its potential to improve animal welfare: a mini review

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Abstract

Castration is frequently used to reduce aggressive behavior and improve the meat quality of animals. Traditionally, surgical and mechanical castration are used to sterilize the animals, but these approaches are associated with a high level of pain, stress, long recovery periods, and post-operative infections. Immunocastration is a new animal-friendly, painless alternative castration technique that is used to prevent undesired sexual behavior, reduce aggressive behavior, prevent unwanted pregnancy, control wildlife populations and wandering species, enhance growth performance, improve meat quality, and treat various sex hormone–dependent disorders. The mechanism of immunocastration includes the immunological block of the hypothalamic-pituitary–gonadal axis (HPG axis) which inhibits gonadotropin secretions, causes atrophy of gonadal tissues, and inhibits gametogenesis, resulting in infertility in both female and male mammals. By the mid-1990s, various immunocastration vaccines have been tested in different animal models to achieve successful castration effects. Recently, genetic immunocastration especially DNA vaccine has gained increasing attention due to its safety, being animal-friendly, and being easy to use. This review aims to evaluate the potential of traditional castration methods, as well as the current status of immunocastration vaccines, their effects, and future prospective.

Keywords Anti-GnRH vaccination · Animal welfare · Castration · Meat quality · HPG axis · Immunocastration

Introduction

Nowadays, the agro-food system is focusing more on the concerns of people and organized activities for the welfare of farm animals, and this caused several corporations to see the costs of investing in a new, sustainable manufacturing paradigm, which covers animal welfare (Bowen 2008). For several centuries, the castration of male animals has been used to control aggressive behavior, reproductive activities, unwanted population, overpopulation, and reproductive diseases, and produce good quality meat production (Pauly et al. 2009; Root Kustritz 2012; Sales 2014). Castration is the process of destruction or removal of gonads to prevent the animals from reproducing. In male animals, it can be achieved through different techniques such as removal of both testicles surgically; mechanically crushing of spermatic cord and testes; destructing of male gonads by chemicals; and lowering the production of sex hormones such as FSH, LH, and testosterone immunologically (Aurich 2018). In females, castration can be accomplished by surgical removal of both ovaries and uterus completely (ovariohysterectomy),

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just ovaries (ovariectomy), and by reducing the hormone levels like LH, FSH, and estrogen by the help of immunological contraceptives (Mancini et al. 2017).

Traditionally, surgical and mechanical castration methods are more common techniques to neutralize the animals. Surgical castration can be accomplished through complete removal of testes, whereas in mechanical method, spermatic cord is crushed to block the blood supply to the testes which causes necrosis of testicles (Ibrahim et al. 2016; Thornton and Waterman-Pearson 1999). However, these traditional methods have been reported to cause pain and infection and are generally not animal-friendly (Brunius et al. 2011). Therefore, meat producers are facing progressive pressure to end the traditional castration due to animal welfare (Garside et al. 2014). Recently, researchers are looking for an alternative non-invasive castration that can potentially substitute traditional castration. In this regard, immunocastration that targets the hypothalamic-pituitary–gonadal axis has been designed and evaluated in different animal species (Karakus et al. 2013; Parthasarathy et al. 2002).

Although no official commercialized immunocastration vaccines are available to date for small ruminants, it has been commercialized, widely used, and has shown promising results of successful castration in the pig industry (Needham et al. 2016). The immunocastration vaccine disrupts the normal function of the hypothalamus to release gonadotropins (GnRH); hence, it impairs the function of the testes and leads to reduced testosterone and sperm synthesis in male animals (Pérez-Linares et al., 2017; Skrlep et al. 2020). This approach aims to block the hypothalamus-pituitary–gonadal (HPG) axis to diminish the pituitary hormonal secretions, sex hormone synthesis, sexual behavior, and testicular growth to attain the castration effect (Han et al. 2015a; Ulker et al. 2009). This review aims to summarize the traditional castration procedures, their limitation and an alternative castration technique—immunocastration—its mechanism, targets, and future prospective.

Methods of castration

The castration of male livestock animals such as cattle, horses, sheep, and goats has been started 8000 years ago (Bowen 2008; Brunius et al. 2011). Depending on the castration purpose, it may be used to attain one or more desirable effects. Occasionally, reversible castration might be required because of possible future reproduction of the respective male and sometimes complete removal of testicles might be desired for irreversible castration of animals. Different conventional and advanced methods for castration have been established with the aim to achieve animal-friendly, safer, efficient, diverse, and cheaper opportunities of restricting the reproductive and testicular functions (Table 1).

Table 1 Different methods of castration with their advantages and disadvantages

Castration methods	Techniques	Mode of action	Advantage	Disadvantage	References
Mechanical method	Burdizzo instrument, latex band, rubber ring	Crushing of spermatic cord above the testes to stop the testicular blood supply	Simple and economical; bloodless; suitable for young animals	Extreme pain; wounds; higher level of operator competence	(Melches et al. 2007; Robertson et al. 1994)
Chemical method	Injection of toxic agents like formaldehyde, lactic acid, silver nitrate	Destruction of sperms and hormone-producing testicular cells; degeneration of testes	Easy to use; produces no harmful effect on animal and administrator person	Expensive; may cause irritation, muscle wasting, and anemia; drug residues	(Capucille et al. 2002; Berlin 2009; D'Souza et al. 2004)
Surgical method	Scalpel, knife, and emasculators	Testes are removed completely through surgical incisions	Sure and irreversible castration; suitable for young animals	Acute pain; risk of infections; blood loss	(von Borell et al. 2009; Bretschneider 2005)
Immunocastration	Immunization by exogenous reproductive hormone or gene vaccines	Blocking the HPG axis; inhibiting sex hormone production and gonad growth	Bloodless; animal-friendly; safe and no pain	Irreversible; requires multiple doses; high price; risk of self-injection	(Wassie et al. 2019, C Dunshea et al., 2001, Ahmed et al. 2022)

Conventional castration techniques

Since then, conventional castrations including surgical castration, chemical castration, and mechanical castration have been used to sterile the animals. In a mechanical method or bloodless castration, a Burdizzo instrument, latex band, or rubber ring is used to crush the spermatic cord above the testes to stop the blood supply to the testicle which leads to necrosis and degeneration of testes. This method is suitable for the castration of young male animals. The Burdizzo method requires a higher level of operator competence, and the animal's discomfort or pain is comparable to other castration methods (Melches et al. 2007; Robertson et al. 1994).

In chemical castration, different substances like formaldehyde and lactic acid have been used for the destruction of sperms and hormone-producing testicular cells in different species (Capucille et al. 2002; Vosoughi et al. 2013). Researchers indicated the advantages of using salts and acids for castration. These substances are not much expensive, easy to use, produce no harmful effect on animal and administrator person, and have a few side effects. Although this castration technique effectively inhibited fertility, it is not suitable for young animals with orchitis and calf during their weaning time. The side effects associated with chemical castration, such as muscle wasting, anemia, osteoporosis, depression, and loss of libido, have been reported in different studies (Berlin 2009; Vinke et al. 2008).

Among conventional castration methods, surgical castration is a common technique in which both testes are excised to make the animal sterile. Previous studies have revealed that surgical castration is a painful method (von Borell et al. 2009; Leidig et al. 2009) and requires skilled labor, and therefore it should be performed using anesthesia and postoperative observation to prevent some complications, such as inflammation, infection, and myiasis (Neto et al. 2014).

Alternatives to conventional castration approaches

Due to the limitations of traditional castration approaches in animal welfare, researchers are intent to find out suitable alternatives. Genetics provides an alternative to surgical castration; multiple genes have been recognized and while genetic selection is not thought to affect the quality of meat, this strategy is possibly a long-term setting of the problem (Brinke et al. 2020; Duarte et al. 2021). In only four generations, androstenone and skatole were anticipated to be selected (Merks et al. 2009). A further strategy to control the production of androstenone and skatole in male animal fats, especially in pigs, can be controlled by a restricted diet (Moore et al. 2017). Hygiene and clean diet can lower the level of skatole (Giersing et al. 2006). To reduce the androstenone, which is associated with behavioral aggression, best management practice should be adopted: keep the pigs of the same age group of male pigs from birth to slaughter with appropriate and additional material to minimize hostile encounters (Bonneau, 2006, Lundström and Zamaratskaia 2006).

The generation of female herds is another alternative to operational castration, but it requires sorted semen that is not available in enough amounts to produce commercial animals and also causes certain problems during pregnancy (Rath and Johnson 2008; von Borell et al. 2009). The sexed semen involved deep insemination and a more invasive technique compared to traditional procedures, which caused pain and danger in female pigs (Giersing et al. 2006). However, recently researchers considered immunocastration as an effective non-surgical alternate (de Roest et al. 2009). In immunocastration, different immunogens are used to break the hormonal balance of reproductive axis (hypothalamic-pituitary–gonadal axis; HPG axis) by targeting the KISS1 and GnRH genes and other reproductive hormones as shown in Fig. 1. In this method, the secretion of GnRH is inhibited, which causes a decline in follicle-stimulating hormone (FSH) and luteinizing hormone (LH) that leads to a decrease in production of sex hormone, functional disruption, atrophy of gonads, and ultimately infertility (Dunshea et al. 2001; Mancini et al. 2017).

Types of immunocastration

Based on immunogenic targets, immunocastration is categorized as gene immunocastration and hormone immunocastration. In hormonal immunocastration, different reproductive hormones (GnRH, FSH, testosterone, LH, etc.) are targeted to reduce their concentration to achieve the castration effects. However, different researches indicated that neutering achieved by the use of reproductive hormones like FSH, LH, or testosterone is not much effective and stable (Schanbacher 1982). Lambs immunized with anti-testosterone treatment can elicit antibodies against testosterone but had no significant impact on serum testosterone level and testicular size (D'Occhio et al. 1987; Walker et al. 1984); it was observed that the administration of LH-RH (luteinizing hormone–releasing hormone) with glycopeptide adjuvant can decrease serum hormones and can induce an immunological neutralization model (Carelli et al. 1985). Likewise, other studies have reported that vaccination against LH can reduce testosterone level and testicular growth, but it has weaker immunogenicity than FSH immunization (Schanbacher 1985, Thompson, 2000). However, some investigators have also found obvious side effects of LH immunization such as alopecia and muscle wastage (Moudgal et al. 1997).

On the other hand, various studies have established more successful and effective immunocastration models through anti-GnRH immunization than pituitary hormones (Doroteu

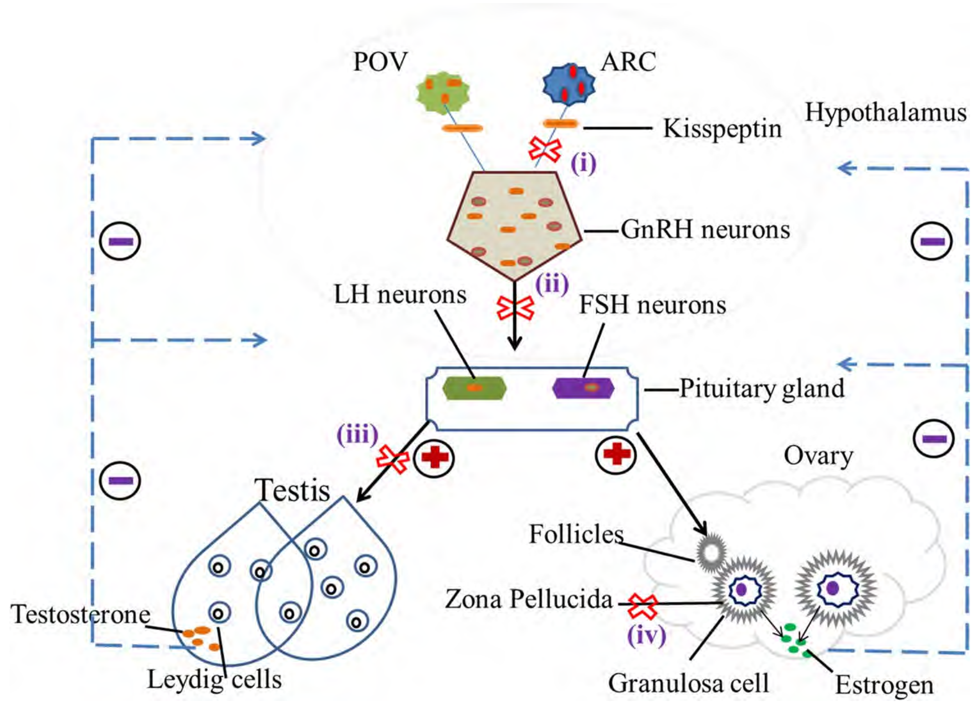

Fig. 1 The mechanism of immunocastration to block the HPG axis in male and female animals. (i) The gene immunocastration vaccines targeting the kisspeptin (KISSI gene vaccines). (ii) The anti-GnRH immunocastration vaccines (Improvac). (iii) The immunocastration vaccines against LH (anti-LHRH). (iv) The contraceptive vaccine targeting the zona pellucida in females to control fertilization (PZP-22)

et al. 2021; Monleon et al. 2020; Oliviero et al. 2016). Anti-GnRH vaccines are used to elicit anti-GnRH antibodies that bind to endogenous GnRH and neutralize its effect (Xu et al. 2017; Zeng et al. 2002). Immunization against GnRH can suppress the hypothalamic secretions of FSH and LH, gonad growth, testosterone production, and spermatogenesis (Lents et al. 2018, Doroteu et al. 2021, Monleon et al., 2020, Rocha et al. 2021). In a castrated model immunized with anti-GnRH, it showed decreased expressions of GnRH receptors in pituitary resulting in reduced LH and FSH secretions, testosterone concentration, and testis size (Han et al. 2015a). Recently, with the progress in genetic engineering technology, a new technology of genetic immunization has been introduced that is performed by injecting a plasmid containing a gene into an animal. This injected gene is taken up by the host immune system and the body produces the specific antibody against the target gene. Gene immunocastration comprises the immunization of a genetic material against the reproductive hormones to block the reproductive activities and functions. The introduction of genetic material induces the immune system to generate a specific antibody against the target gene product that is neutralizing the endogenous reproductive hormones and disrupts the reproductive axis to achieve castration goals (Han et al. 2018; Han et al. 2016; Miller et al. 2000).

Hormone-based immunocastration vaccines

The target of hormone-based immunocastration includes some reproductive hormones and regulatory genes such as FSH, LH, and GnRH.

Vaccines against GnRH

The GnRH decapeptide is present in both genders (males and females) and its structure is conserved in all animals. The vaccination against GnRH can be used in both genders. Anti-GnRH vaccines can be utilized to limit sexual behavior, reproductive activities, and sex steroid hormones in companion (dogs) and meat production animals (pigs, sheep, goat, bulls). Since the growth of accessories such as the prostate and the breast is supported by sex hormones, anti-LHRH therapy is appropriate in sex hormone disease (Talwar 1999; Talwar et al. 2004).

Isomers of GnRH

Two more isomers of lower vertebra origin exist in human beings including the traditional decapeptide (pGlu-His-Trp-Ser-TyrGly-Leu-Arg-Pro-GlyNh2) currently represented as GnRH-I. The amino acid GnRH-II, which is originally chicken extracted and has three peculiarities in GnRH-I, is also found in the human brain (White et al. 1998). GnRH-III is also present in humans which was first detected in salmon (Yahalom et al. 1999). GnRH-II gene code is found in humans on the 20p13 chromosome and is separate from the GnRH-I gene on the 8p21-p11.2 chromosome. Each isoform has a specific place in the brain, showing different evolutionary origins and/or functions (Urbanski et al. 1999). The physiological role of GnRH-II and GnRH-III is not determined in humans and other mammals. Hypothalamic GnRH-1 is the primary hormone of the hypothalamic-hypophysical-gonadal axis in addition to a likely direct action in extra-pituitary organisms such as testis, prostate, and placenta (Yahalom et al. 1999). Most studies are focused on GnRH-I, commonly known as LHRH or GnRH.

Semi-synthetic GnRH vaccines

GnRH-I is a small decapeptide that is present in the animal body; thus, it may recognize self-antigen by the immune cell. Therefore, to induce immunity against it, it must be combined with a carrier or adjuvant to activate the T-helper cells (Jinshu et al. 2004). In the absence of a functional connection group, the GnRH-1 analog was presented, in which the D-lysine replaces glycine at position 6, generating a replacement NH2 group that could be safely attached to a carrier such as DT via a spacer (Talwar 1999). Studies have shown that immunization of rats with GnRH-6-DLys-DT vaccine caused atrophy of the prostate and reduced testosterone, thereby inhibiting fertility (Giri et al. 1991; Jayashankar et al. 1989; Jinshu et al. 2004). Similarly, the effect of this vaccine on the inhibition of prostate tumor cell proliferation in rats has been recognized (Fuerst et al. 1997; Rovan et al. 1992).

Vaccines for animal welfare and improvement of meat quality

Immunocastration is now considered an animal-friendly technique to produce good quality meat, free of aroma, and to control the wild and unwanted population humanely. It has been reported that androgen hormones, androsterone, and skatole that accumulate in the adipose tissue can cause undesirable meat flavor. Immunocastration has been reported to improve meat quality by reducing these hormones. For example, immunization of animals with anti-GnRH vaccine could reduce testosterone and remove the taint, thereby enhancing meat quality (Cronin et al. 2003; Dunshea et al. 2001). Similarly, Oliver et al. (2003) reported that immunization of boars and gilts with Improvac (GnRH) and porcine somatotrophin vaccination had a synergistic effect to improve growth performance and inhibit fertility. On the contrary, some scholars reported that castration has a negative effect on fattening and meat composition (Erol and Ünal 2021), whereas another study documented that immunization of goats with Kisspeptin-54 DNA vaccine did not affect meat quality (Wassie et al. 2020). In addition to its use for livestock, anti-GnRH vaccination has been used to reduce wild animal populations with good results (Curtis et al. 2002; Kirkpatrick et al. 2011; Levy 2011; Miller et al. 2000).

Recombinant anti-GnRH vaccines

A growing body of evidence has shown that GnRH-I vaccine can be used without negative effects on people and animals. These vaccines are useful in prostate patients' treatment and are effective in reducing wild animal fertility by inhibiting the production of sex steroid hormones, hence regulating the estrus and libido of meat animals. The production of recombinant vaccinations on an industrial scale would be considerably cheaper than synthetic vaccines. Hsu et al. (2000) combined the receptor-binding domain of Pseudomonas exotoxin A with several copies of GnRH and produced high antibody titers with ovarian atrophy in rabbits. This immunization is advisable in treating GnRH-sensitive ovarian cancer.

Another researcher designed a vaccine with three GnRH repeats conjugated with human IgG1 and T-helper peptides of the measles virus. The DNA coding region of this complex design was attached to C-terminal of asparaginase-produced immunogenicity (Jinshu et al. 2004). The repeated sequence of GnRH vaccine has been found effective to reduce testosterone production to achieve castration along with prostate atrophy in rats (Talwar et al. 2004). In the design of this vaccination, four or five T-non-B-cell peptides interspersed in four or five LHRH units have been substituted by DT/TT as the carrier utilized in earlier semi-synthetic vaccines. This was done to prevent carrier-induced epitope deletion of DT/TT carrier conjugates (Sad et al. 1991) and to interact with MHC throughout the spectrum in a polygenetic population employing a range of these T-cell determinants. The genes have been assembled, cloned, and highly expressed in *E. coli* (Gupta et al. 2004). Using a pH 3 buffer, protein can be extracted from included bodies with modest levels of chaotropic chemicals (2 mol/l urea rather than 8 mol/l). The protein in the immune system has been improved and regenerated (Raina et al. 2004). A similar approach was used to link GnRH with four promiscuous T-helper sequences and, in some circumstances, with the adjuvant Yersinia invasion peptide. The over-production of antibodies against (DT/TT) carrier is prevented, and antibodies against T-non-B-cell peptides utilized as carriers are not anticipated (Finstad et al. 2004).

Gene immunocastration vaccines

With the help of genetic engineering techniques, researchers are able to construct genetic (DNA) immunocastration vaccines for animal use. Different commercial veterinary vaccines that have been developed for fertility control of male and female animals are available in the market (Table 2). In recent past, different studies have been conducted to construct the immunocastration gene vaccine against GnRH. The results proved that these vaccines have an ability to produce antibodies against endogenous GnRH that inhibit the

Table 2 The available commercial immunocastration and immunocontraceptive vaccines

Vaccine names	Targeted animals	Doses	Indications	References
PZP-22	Mares, deer	Two doses at a month apart and annual booster	Fertility control	(Carey et al. 2019)
ZonaStat-H	Wild animals, equine	2–4 doses with 30-day intervals and annual booster	Fertility control	(Lyda et al. 2005)
SpayVac	Equines, deer	Single dose	Fertility control	(Roelle et al. 2017)
Vaxtrate	Bovine, bucks	Two doses with 2-week intervals	Fertility control	(Godfrey et al. 1996)
Gonancon	Deer, dogs, cats, badgers, horses	Single dose	Contraception	(Cowan et al. 2019; Miller et al. 2008)
Bopriva	Cattle	Two doses with 3-week intervals	Immunocastration	(Hirsbrunner et al. 2017)
Improvac	Swine	Two doses with 4-week intervals	Immunocastration	(Wang et al. 2019; Zoels et al. 2020)
Equity	Mares, dogs, and deer	Two doses with 4-week intervals and booster dose every 6 months	Population and estrus control	(Siel et al. 2020)
Repro-BLOC	Elephant	Priming dose and booster dose quarterly	Contraception	(Boedeker et al. 2012)

GnRH secretion and thus reduce the level of FSH and LH to achieve the goal of castration. Mostly scientists used two approaches (DNA-encoded and protein-conjugate analogue vaccines of desired genes) to block the activity of the hypothalamic-pituitary–gonadal axis (HPG axis), which inhibits the reproductive steroid hormones and achieve castration effect. DNA immunization is a novel method to produce antibodies against targeted genes. It is much more effective and cost-effective than recombinant protein vaccines.

Immunocastration with DNA vaccines that achieved a successful castration effect is well documented in various studies. In this regard, Khan et al. (2008) constructed a DNA vaccine encoded with GnRH gene that reduced the level of testosterone, decreased sperm concentration, arrested spermatogenesis, and impaired fertility. In another study, Han et al. (2016) used a GnRH DNA vaccine on mice and found a high level of anti-GnRH antibody and reduced spermatogenesis. These studies highlighted the effectiveness of GnRH gene vaccines to produce anti-GnRH antibodies and reduce sexual activities to achieve a castrated model.

After the discovery of the *KISS1* gene as a novel tumor metastasis suppressor gene in malignant melanoma cells, it is recognized that the *KISS1/GPR54* system has a prominent effect on GnRH secretion, which regulates the activities of HPG axis in females and males. The KISS1 gene is located at the upper end of the HPG axis. It is widely expressed in tissues such as the placenta, brain, and testis, and its expression product kisspeptin is produced by cleavage. Kisspeptin is a product of KISS1 that is cleaved to produce a biologically active polypeptide (kisspeptin-54) containing 54 amino acids. Kisspeptin-54 can be further cleaved into kisspeptin-14, kisspeptin-13, and kisspeptin-10. The RF amide binds to G protein-coupled receptor 54 (GPR54) in cells such as hypothalamic cell lines, pituitary cells, and testicular cells, thereby activating the HPG axis (Kotani et al. 2001). Recent studies on humans and other animals such as mice, sheep, and cattle indicated that the initiation of puberty is also regulated by KISS1 gene by the activation of gonadal function (Bo et al. 2020; Decourt et al. 2016). Similar evidences are also present in other studies that specify the role of KISS1 gene in the development of gonads, ovulation, and puberty. In a mice model, a kisspeptin injection before the onset of puberty can enhance the puberty development (Decourt et al. 2016; Uenoyama et al. 2015). Due to upstream location of KISS1 gene in the HPG axis and its role in reproduction, development of immunocastration vaccine by targeting the KISS1 gene may achieve better results. Previous studies revealed that KISS1 gene vaccines can break the HPG axis and reduce the level of sex hormones and controlled sexual behavior in different animals (Han et al. 2015b; Tesema et al. 2017; Wassie et al. 2019). In a current transcriptome study, the role of KISS1 gene vaccine in the cellular immunity of testes has been identified. In this study, the transcriptomic analysis of ram testes is used to identify the cell-mediated immunity induced by KISS1 gene vaccine. The results showed that the immunized animal group has increased differentially expressed genes related to hormonal regulation, immune response, spermatogenesis, and gonad development. The vaccinated group also has the highly expressed up-regulated genes related to regulation and activation of cell-mediated animal response (Ahmed et al. 2022).

For female animals, which are generally referred to as contraception rather than castration, immunocontraception uses immunological targets similar to those of males. The only difference is that in females, estrogen and zona pellucida (ZP) can be targeted to develop contraception. Estrogen therapy or knockout of the estrogen receptor gene leads to decreased fertility and altered sexual behavior in females (Goyal et al. 2003; Ogawa et al. 1997). The zona pellucida is a non-cellular structure that is coated outside the oocyte and outside the pre-implantation embryo. During sperm-egg combination, the sperm identifies a specific site on the zona pellucida on the surface of the egg, which in turn induces a sperm acrosome reaction through the zona pellucida, which then fuses to form a fertilized egg (Wassarman 1999). ZP3 is generally considered to be the first receptor for sperm, ZP2 is the second receptor for sperm, and ZP1 acts as a link between ZP3 and ZP2. Immunization of marmosets with porcine ZP3 caused long-term infertility (Barber and Fayrer-Hosken 2000).

Immunization program

A minimum of two doses are necessary to diminish the size of the testes and the accessory reproductive organs and to remove meat taint in pigs (Brunius et al. 2011; Cronin et al. 2003; Doroteu et al. 2021; Moore et al. 2017; Zamaratskaia et al. 2008). Immunocastration enables boar-like growth potential to be exploited until the second immunization succeeds in castration effect. The primary vaccination primes the animal's immune response and the second dose increased the antibody titer. Different researchers achieved the castration effect by injecting multiple doses of antigen. Ferro et al. (2004) immunized the rats at fortnightly intervals with GnRH-I peptide and observed arrested spermatogenesis and testicular growth. Another study indicated that immunocastration with Bopriva on days 1, 21, 101, and 181 has shown to be effective to decrease the level of testosterone, aggression, and sexual behaviors on Holstein bulls (Bolado-Sarabia et al. 2018). Lents et al. (2018) revealed that goats immunized with two doses of anti-GnRH conjugate vaccine at 8 months of age with 30 days apart successfully reduced testicular size, seminiferous tubule circumference, and seminal production. Immunocastration of pigs with Improvac at the age of 79 and 142 days caused a significant elimination of boar taint and reduced the growth of reproductive organs (Skrlep et al. 2012). Likewise, previous researches reported the immunology and reproductive effects of subcutaneous injection of GnRXG/Q recombinant immunocastration vaccine in dogs on days 1 and 30. The results were proved that there was a significant increase in IgG and reduction in testosterone up to day 270 (Donovan et al. 2012; Liu et al. 2015). Similarly, another study conducted on bulls suggested that two doses of immunocastration antigen can induce the production of antibodies causing the GnRH to subsequently create a barrier between the hypothalamus and anterior pituitary gland which blocks the production of GnRH-I, LH, and testosterone (Herbert and Trigg 2005, R Huenchullan et al. 2021).

In addition to the multiple immunizations, scientists tested the single-injection immunocastration to minimize the cost and efforts. A single-injection immunocontraceptive vaccine aiming at the gonadotropin-releasing hormone in women has been shown in recent studies for a long time to exhibit infertility. The results of the previous study demonstrated an immediate effect on the fertility indicators evaluated and reduced subsequent cubic production in one single GonaCon injection on badgers in the following year. The authors said it would be necessary to administer the vaccination at least every 2 years to maintain female infertility levels (Cowan et al. 2019)**.** Correspondingly, Miller et al. (2008) immunized black-tailed deer with GnRH-KLH vaccine conjugated with an adjuvant, AdjuVac, in a single shot. The results indicated that AdjuVac is important for the effectiveness of the single-dose GnRH vaccine that can decrease the pregnancy rates in deer (Miller et al. 2008). However, in male animals, single injection is not effective to achieve long-term castration effect. Ajadi and Oyeyemi (2015) concluded that a single dose of GnRH vaccine caused testicular changes, reduced the semen quality, and caused infertility up to 16 weeks of vaccination (Ajadi and Oyeyemi 2015). The modifications in antigen delivery, use of adjuvant, and drug release rate can enhance the immune response of target animals. Mice vaccinated with a single subcutaneous injection of GnRH-MAP coupled with an implant containing GnRH-MAP dendrimer polyanhydride enhanced the antibody titer and produced humoral and cell-mediated immunity up to 41 weeks after primary immunization.

Conclusion and future direction

Immunocastration targets hormones, genes, and receptors in HPG axis to achieve the castration effect. Targets located upstream of the HPG axis, such as KISS1and GnRH, are better at reducing sex hormone levels and inhibiting reproductive function. Generally, immunocastration is effective, safe, animal-friendly, and suitable for animals of all ages, so it can be the best alternative to other castration techniques. In summary, this review provides useful information for the application of immunocastration to a wider range of fields by summarizing the characteristics, target selection, safety, and reversibility. Although immunocastration has been tested in animal models and humans, there is no effective functional castration for the treatment of sex hormone dependence disease. Thus, effective immunocastration that inhibits sex hormone dependence disease should be investigated in the future. Prospective studies should be focused on the effect of immunocastration testicular germinal epithelium and reproductive behavior and long-term effect with the least number of antigen administrations.

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Data availability All data are freely available to use for non-commercial purposes.

Declarations

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Consent to participate All authors have read the manuscript and contributed to manuscript revision and formatting.

Consent for publication All authors have agreed to submit this article for publication.

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