Theriogenology 89 (2017) 329–337

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Review

Ovarian dysfunction associated with zona pellucida–based immunocontraceptive vaccines

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ARTICLE INFO

Article history:
Received 1 June 2016
Received in revised form 9 September 2016
Accepted 9 September 2016

Keywords:
Porcine zona pellucida
Contraception
Anestrus
Oophoritis
Freund's adjuvant
Cytotoxic T-lymphocyte

ABSTRACT

Despite more than 40 years of research into zona pellucida (ZP)–based vaccines, relatively little is known about their mechanism of action. Early research demonstrated precipitation of ZP glycoproteins by antiovarian antiserum, rendering oocytes resistant to sperm binding *in vitro*. Subsequent work showed significantly decreased fertilization rates following passive immunization, sparking interest in anti-ZP immunocontraception for human and animal use. The primary mechanism of action of ZP vaccines is generally considered to be an antibody-mediated interference with sperm–oocyte binding and/or fertilization. However, this mechanism of action excludes the potential for ovarian dysfunction associated with anti-ZP treatment in some species. A review of relevant literature in pertinent model, domestic and wildlife species reveals a variety of previous and current hypotheses for ovarian effects following ZP-based immunization. Ovarian dysfunction has been suggested to be a species-specific response. In addition, cytotoxic T-lymphocytes and the use of Freund's adjuvants have been suggested to play a role. Finally, the type and extent of glycosylation of ZP antigens have been proposed to influence ovarian effects. The validity of these hypotheses is re-examined in the light of current knowledge. Further investigation of ovarian function in species believed to be resistant to the ovarian effects of anti-ZP vaccines is warranted. To this end, anti-Müllerian hormone may provide a novel tool for the assessment of ovarian function during ZP-based immunocontraception, particularly in wildlife species not amenable to frequent clinical examination.

1. Introduction

The zona pellucida (ZP) is a complex glycoprotein matrix surrounding the mammalian oocyte and early conceptus. Comprised of either three or four glycoproteins, the ZP plays a pivotal role in the union of oocyte and spermatozoon during mammalian fertilization, arguably the most important joining event in biology. In addition, the ZP functions in the induction of the acrosome reaction, the prevention of polyspermy, and protection of the early embryo [1]. Furthermore, the ZP is intimately involved in communication between the oocyte and its surrounding granulosa cells in the developing follicle [2]. These critical functions of the ZP in reproduction and its tissue-specific nature have encouraged research into its role as an immunocontraceptive for over 40 years [3].

Porcine zona pellucida (pZP) with added adjuvant remains the most common native form of the vaccine due both to the homology between the ZP proteins of many mammalian species and its availability in relatively large quantities [4,5]. Approximately 80 zoo and wildlife species have been successfully contracepted using pZP [6]. Despite this widespread application, relatively little is reported describing the vaccine's mechanism of action. In research

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0093-691X/$ – see front matter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2016.09.018

aimed at humans, initial enthusiasm for ZP-based immunocontraception waned sharply following reports of ovarian dysfunction in rabbits and nonhuman primates [7,8]. A number of hypotheses regarding the causes of ovarian dysfunction during ZP-based immunocontraception have since evolved.

This review revisits initial studies describing ovarian tissue, oocytes, or ZP as immunological agents, on which our current understanding of pZP's mechanism of action is based. In addition, several hypotheses advanced to explain ovarian dysfunction observed during ZP-based immunocontraception are re-evaluated based on relevant literature reporting on common laboratory and domestic animal species, as well as the feral horse, deer, and African elephant.

2. Early work on ZP antigens and antisera: a journey down the memory lane

Interest in immunological methods of fertility control dates back to the late 1890's with an initial focus aimed primarily at testes and spermatozoa as immunizing agents, reviewed by Tyler [9]. Reports of antisera to ovarian homogenates blocking fertilization processes in the sea urchin [10] and frog [11] encouraged interest in the mammalian oocyte and ovary as putative antifertility antigens. Initial studies demonstrated the existence of organ-specific antigens in the guinea pig ovary and testis [12,13]. Early immunofluorescence studies further localized common antigens to the ZP, atretic follicles, and the acrosome of spermatozoa [14].

Ownby et al. [15] injected golden hamster ovarian homogenates combined with Freund's complete adjuvant (FCA) into rabbits. Boosters, consisting of ovary homogenates with Freund's incomplete adjuvant, were followed by weekly serum sampling. The antisera produced included antibodies to at least one antigen unique to the ovary, demonstrated using agar-gel diffusion plates. Superovulated hamster eggs exposed to rabbit antiovary antisera formed a precipitate in the ZP that was visible under light- and phase-contrast microscopy. The precipitated ZP was found to be resistant to digestion by trypsin. Similar findings were reported by Sacco et al. [16].

Antiovarian antiserum, added to hamster ova before exposure to homologous spermatozoa *in vitro*, interfered with sperm–oocyte binding. None of 170 pretreated oocytes was penetrated by spermatozoa, in comparison to nearly half of 58 control oocytes [17]. The use of homogenized oocytes rather than the whole ovary as an immunogen produced similar results [18]. Despite ZP-based immunocontraception being in its infancy, researchers noted the potential advantages of this method of fertility control, including reversibility and the absence of somatic side effects because of the specificity of anti-ZP antibodies [17].

Jilek et al. [3], using mice passively preimmunized with rabbit anti-mouse ovary antisera, demonstrated via immunofluorescence the presence of anti-ZP antibodies bound to oocytes aspirated from antral follicles, as well as ovulated oocytes. This showed that anti-ZP antibodies were capable of reaching the ZP *in situ* within the follicle. In addition, passive immunization was found to decrease fertilization rates from over 91% to below 1%. The authors concluded that the effect on fertility *in vivo* "seems to be a block to sperm penetration through the ZP, on which antibodies were actually detected within the follicles".

Further study of aspirated oocytes and early embryos flushed from the uterus or uterine tubes of untreated hamsters showed that antihamster ovary antiserum precipitated the ZP of preovulatory oocytes as well as early embryonic stages *in vitro*. In addition, precipitation of the ZP following fertilization was thought to inhibit the attachment of transferred embryos to the endometrium [19], possibly as a result of interference with embryonic hatching [20]. In a similar study in mice, antioocyte and anti-ZP antisera had no effect on the development of early embryos to the blastocyst stage *in vitro*, although a small effect on zona shedding was noted [21]. A later study in the same species found that preincubation with anti-ZP antiserum had no effect on early embryonic development and zona hatching *in vitro*, nor implantation and further development of pretreated embryos transferred to pseudopregnant recipients, despite visible precipitation of the zonae [22]. Similarly, mice passively immunized with anti-ZP antiserum 2 days after mating showed no adverse effects on fertility or fecundity, and anti-ZP antisera had no effect on early embryonic development *in vitro* [23].

From these initial studies and those that followed, hypotheses regarding ZP vaccines' mechanism of action evolved as a primarily antibody-based interference with one or more of the following processes: sperm–oocyte binding, the acrosome reaction, sperm movement through the ZP, oocyte activation and/or the zona block; thus, at the level of the periovulatory oocyte. If so, estrous cyclicity and reproductive behaviors should remain unaffected following vaccination. This feature of pZP immunocontraception has been an important, although occasionally controversial, rationale supporting its application in species with complex social hierarchies, such as the African elephant and feral horse [24–28]. The detection, however, of ovarian dysfunction subsequent to treatment with ZP-based vaccines in some species has provided a challenge to researchers hoping to overcome this potentially undesirable outcome. During this process, a number of hypotheses regarding the cause of ovarian dysfunction have developed.

3. Hypotheses explaining ovarian dysfunction during ZP-based immunocontraception

3.1. Glycosylation causes oophoritis

Glycosylation refers to the pattern of binding of distinct carbohydrate moieties to amino acids, giving rise to the glycoprotein structure of the ZP. The diverse nature of ZP glycosylation across species may play a role in the species-specificity of sperm-zona binding [29]. Chemical deglycosylation of pZP3 was found to decrease its antigenicity and immunogenicity; precipitation of ZP *in vitro* by the relevant antisera was more superficial than precipitation produced using antisera to glycosylated pZP3 [30]. Consequently, early workers in the field considered the deglycosylation of ZP antigens to negate pZP's oophoritogenic effects. In rabbits, the degree of glycosylation of pZP was found to correlate directly with the degree of interference with