

Occurrence of Anti- Gonadotropins Hormones Antibodies in Rabbits Immunized with *Pseudomonas Aeruginosa*

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Abstract

The roles of gonadotropins hormones in fertility are well documented. This report deals with the effect of rabbit anti-*Pseudomonas aeruginosa* immune sera binding and depleting human Leutinizing hormone (LH) and follicle stimulating hormone (FSH). Capturing ELISA (Pull-down assay) was used to enumerate the depletion potential of immune sera. The finding indicated the presence of anti- hormones antibodies in anti-bacteria immune sera. Percent depletion of males anti-bacteria antisera for (LH) was 35.1 ± 9.6 in test versus 3.3 ± 5.8 control ($P=0.001$) and for (FSH) was 38.95 ± 7.96 in test versus 2.1 ± 1.70 in control ($P=0.0001$).

On the Other hand, the percent depletion of female anti-bacteria antisera for LH was 10.9 ± 16.9 in test versus 0.6 ± 1.07 in control, and for FSH was 14.04 ± 21.79 in test versus 1.075 ± 1.07 for control.

The presence of anti-gonadotropins hormones antibodies generated through immunization with *Pseudomonas aeruginosa* implicates a role of the immune response to this bacterium in immune-mediated effect in human fertility process.

Keywords: Anti - Gonadotropins, Anti-Hormone antibody, fertility

Introduction

Gonadotropins exert a highly controlled effect on male testes and female ovary, through hypothalamic-pituitary-testes or ovary axes respectively.

Dysregulation of these axes could be affected by immune system and involvement of autoimmune processes in anti-gonadotropins effect is well documented (Fenichel *et al.*, 1999; Gleicher *et al.*, 1987; Forger *et al.*, 2004; Reimand *et al.*, 2001).

Among the documented anti-gonadotropins, FSH blocking antibody was demonstrated in premature ovarian failure (POF), polycystic ovary syndrome (PCOS) mediated by anti-FSH antibody (Haller *et al.*, 2005) and Recurrent abortion associated with anti- B_2 - GP_1 antibodies. Many other infertility associated autoimmune-mediated condition is reported (Haller *et al.*, 2012). Infection by diverse species of bacteria has effect on reproductive function, among the constituent of gram negative bacteria is lipopolysaccharide (LPS) which was shown to suppress, through immune inflammatory challenge the level of LH (Alam *et al.*, 2014).

The precise role played by bacteria is not completely understood. This communication deals with the effect of immune response to a Gram negative *Pseudomonas aeruginosa* on generating a cross-reactive anti-gonadotropins antibodies that affect LH and FSH.

Materials and Methods

Animals

Rabbits 4-6 months old of both sexes were used in the preparation of anti-*Pseudomonas aeruginosa* antisera. They were housed in pair per cage and fed ad libitum. They were ethically treated according to the established guideline in our department.

Immunization

Animals were immunized with whole heat killed bacteria following the procedure of (Duncan *et al.*, 1976) except using subcutaneous route instead of intravenous route. Control non-immunized rabbits were included.

The animals were rested for two weeks before harvesting their sera while they are under ketamine anesthesia. Sera were kept at $-10C^{\circ}$ until used.

Human gonadotropins

Normal mature human female sera were obtained seven days after the beginning of the menstrual cycle. The levels of LH and FSH were estimated using commercial ELISA Kits. These levels represent values before capturing.

Capturing ELISA

The same ELISA Kits used as stated above were used.

The procedure followed was a modification of the procedure of (Tsai *et al.*, 1987) and as follows :

Coating

It involved dilution of rabbits antisera to 1/10 with saline. Wells of flat bottom 96-well microtitration plate were coated with 200 μ L of each sera using sera diluted 1/10 in 0.1 M bicarbonate buffer PH 9.0. The coated plate were left at 37C° for 2 hours and at 4C° overnight, washed three times with washing solution containing PBS and 0.05% Tween 20 (washing buffer). Blocking was with 1% bovine serum albumin in coating buffer. Coating was at 37C° for one hour. Washing was repeated.

Capturing

Hormones were captured by adding 200 μ L of hormone sample (Human female sera) to antibody coated wells, followed by incubation at 37C° for one hour with frequent agitation. The sample was removed and assayed for the hormones levels as outlined above.

Depletion

Percent depletion was calculated using the formula

$$\frac{\text{Hormone level before depletion} - \text{Hormone level after depletion}}{\text{Hormone level before depletion}} \times 100.$$

Statistical analysis

The level of significance between test and control means were calculated using t-test using "Statistics for Epidemiology" program.

Results

The findings presented in (table 1). showed that most rabbits immunized with *Pseudomonas aeruginosa* demonstrated anti-LH and anti-FSH antibodies in comparison to control non-immunized rabbits.

On the other hand however, 2 out of 6 of female rabbits showed the presence of anti-LH antibodies in their sera- and a similar number showed anti-FSH antibodies as well. None of the control male and female rabbits showed any anti-hormone antibody. Capturing ELISA was used to evaluate pulling-down of LH and FSH hormones by rabbit anti-bacterium-immunized males, the percent of depletion of (LH) hormone was 35.1 \pm 9.6 versus 3.3 \pm 5.8 in control animal (P=0.001) similar findings were obtained for FSH and the percent depletion 38.95 \pm 7.96 in test immunized animals compared to 2.1 \pm 1.79 in control non-immunized animal

(P= 0.0001). In Females immune sera (table 1). the depletion of LH and FSH were 10.95 \pm 16.9 and 14.04 \pm 21.79 compared to control values of 0.62 \pm 1.07 and 1.075 \pm 1.075 respectively (P=0.34 and 0.35 for LH & FSH respectively).

Table 1. Anti-gonadotropins antibodies in male and female rabbits immunized with *Pseudomonas aeruginosa* compared to controls.

Anti-Hormone antibody (No.Positive/total)			
Treatment	n	LH	FSH
Male			
Test	7	6/7	7/7
Control	3	0/3	0/3
Female			
Test	6	2/3	2/6
Control	3	0/3	0/3

Table2. Rabbits males and females anti- *Pseudomonas aeruginosa* immune sera capability of depleting human gonadotropins.

Treatment	n	% Depletion (Mean \pm SD)		P-Value	
		LH	FSH	LH	FSH
Males					
Test	7	35.1 \pm 9.6	38.95 \pm 7.96	0.001	0.0001
Control	3	3.3 \pm 5.8	2.1 \pm 1.79		
Females					
Test	6	10.9 \pm 16.9	14.04 \pm 21.79	0.34	0.35
Control	3	0.62 \pm 1.07	1.075 \pm 1.075		

Significant was regarded by $P \leq 0.05$.

Discussion:

Anti-gonadotropins antibodies were demonstrated in diverse conditions related to infertility (Haller *et al.*,2012). Immunization of rabbits of both sexes induced an immune response directed at LH and FSH hormones (Table 1 and 2). In this regard, *Pseudomonas aeruginosa* has lipopolysaccharide, the main constituent of its O-antigen, its sugar composition might furnish an antigenic determinant (Forger *et al.*,2004) that might generate a cross-reactive immune response to the glycoproteins hormones under study.

Molecular mimicry, is an open, attractive mechanism for initiating auto- immunity (Alam *et al.*,2014). Regarding a molecular mimic cross-reactive immune response, LH and FSH are glycoproteins, their glycan could be cross-reactive with bacterial constituent. In this instance, Anti FSH antibody is a natural antibody in healthy women (Haller *et al.*,2012) and humoral memory associated with these antibody, mediated by B cell might contribute to a cross-reactive autoimmune response that is directed to these glycoproteins (Binder&Silveman, 2005).

The data presented in (table 2). show that part of LH or FSH are depleted which implicate certain epitope that could be shared by the two hormones and reactive with anti-bacteria antisera .

The increased percentage of antibody capturing the hormones is interesting , nevertheless, coated antibody could not operate fully due to conformational constrain due to attachment of the antibody to polystyrene surface. This implies that more depletion could be mediated by the free antibody present in the immune sera. Anti gonadotropins antibodies may impair the function of exogenous or endogenous FSH , Anti-FSH antibody could form immune complexes with FSH mediating it's clearance as shown for creatine kinase in patient with corresponding antibody (Warren *et al.*,2006). Simillar situation could exist for LH and its antibody. The immunopathophysiology exerted by such bacterial induced anti-gonadotropins immune response is noteworthy and requires indepth investigations.

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