IMMUNE RESPONSE TO REPEATED rhFSH SUPEROVULATION TREATMENT IN RABBIT DOES

Cortell C., Viudes de Castro M.P.*

Centro de Tecnología Animal, Instituto Valenciano de Investigaciones Agrarias (CITA-IVIA), Polígono de la Esperanza, 100, 12400 Segorbe (Castellón), Spain
*Corresponding author: mpviudes@ivia.es

ABSTRACT

Some studies have demonstrated that, when superovulation is induced more than once in the same animal, the response to treatment may be reduced. This reduced response may be related to an increase of anti-gonadotrophin antibodies. The aim of this study was to evaluate the effect of repeated recombinant human (rh) FSH superovulation treatments on the ovulation rate and anti-FSH antibodies production. For this purpose, 34 females were treated i.m. with rhFSH (0.6 μg every 24 h, for 3 days) in order to induce superovulation four consecutive times. Control does were injected with the vehicle at the appropriate time. The interval between the first three treatments was one month; and three months between the third and fourth treatment during which the females were inseminated without superovulation treatment. Ovulation rate was checked by laparoscopy and blood samples were collected after each treatment. An indirect ELISA was used to detect sera anti-FSH antibodies. The ovulation rate was significantly higher in does treated with rhFSH than in control group. The ovulation rate was significantly higher in does treated for the first time with rhFSH than in those treated two, three, or four times (8.7±1.42, 19.3±1.36, 13.5±1.26, 13.0±1.28, 14.3±1.31 for control and superovulated females, respectively, $P<0.05$). On the other hand, results obtained after four consecutives rhFSH treatments indicate that there was a significant difference in immune response of does after the second treatment ($P<0.05$), none of the treated females presented immune response in the first or second treatment, on the contrary, in the third and forth treatment the 40 to 60% of females presented high antibody levels. The results of the present study clearly demonstrate that repeated rhFSH superovulation treatments in rabbit does induce a immune response and have a negative effect on ovulation rate. Although anti-FSH antibodies induce a decrease in superovulation response, the ovulation rate of females superovulated twice, three and four times was significantly higher than control females. The immune response developed has an important individual variability and may be related with the reproductive response decrease after repeated treatments. Nevertheless, since there were females in which ovulation rate diminished without an increase in sera antibodies, it is clear that reproduction failure after consecutive superovulation treatments can be caused by different reasons, which have to be studied in future.

Key words: Superovulation, Immune response, Ovulation rate, rhFSH.

INTRODUCTION

Rabbit is widely used for biomedical purposes in several areas. Genetic lines for high growth rate, litter size, and fur quality have been developed associated to reproductive technologies for increasing the productivity (Besenfelder *et al.*, 2000; Garcia and Baselga, 2002; Vicente *et al.*, 2003; Baselga, 2004; Gondret *et al.*, 2005).

The use of reproductive technologies requires the highest efficiency, and thus, when gamete recovery is being planned, a superovulation treatment is usually used in order to obtain the highest number of them. In rabbits, both FSH (Mehaisen *et al.*, 2006; Salvetti *et al.*, 2007) and eCG (Mehaisen *et al*,. 2005; Mehaisen *et al.*, 2006) have been used to increase the ovulation rate. However, superovulation may cause some problems including increase in the number of haemorrhagic follicles or decrease in

the quality of embryos (Chrenek *et al.*, 1998; Kauffman *et al.*, 1998; Mehaisen *et al.*, 2005; Salvetti *et al.*, 2007).

On the other hand, when embryos are recovered from rabbits with high genetic value, the chance of recovering more than once from the same female is interesting for maximizing the number of embryos or oocytes collected (Medjdoub *et al.*, 2000; Forcada y López, 2000, Mehaisen *et al.*, 2004). Nevertheless, some studies have demonstrated that, when superovulation is induced more than once in the same animal, the response to treatment and gametes quality may be reduced in rabbits (Mehaisen *et al.*, 2006) and in other species as mice (Van Blerkom and Davis, 2001; Combelles and Albertini, 2003) and cats (Swanson *et al.*, 1996). This reduced response may be related to an increase in anti-FSH or anti-eCG sera antibodies (Boiti *et al.*, 1995; Swanson *et al.*, 1996).

The purpose of this study was to evaluate the effect of repeated rhFSH superovulation treatments on the ovulation rate and anti-FSH antibodies formation.

MATERIALS AND METHODS

Animals

Thirty-four rabbit does from a maternal line were used. Animals were kept individually in flat deck cages under a photoperiod of 16 light:8 dark hours. Females were fed with a commercial diet *ad libitum* and were housed at the experimental farm of the CITA-IVIA (Segorbe, Castellón).

Superovulation treatment and blood samples collection

Superovulation treatment was induced using recombinant human FSH (Gonal-F® 75, Serono, Italy). Females were treated intramuscularly, every 24 hours, during three days with 0.6 µg of rhFSH dissolved in 0.4 ml of Polyvinylpirrolydone 30% (PVP; Sigma, St Louis, MO, USA). In the control group, females were injected with the vehicle at the appropriate time. The interval among the first, second and third treatment was one month; while three months passed between the third and fourth treatment. In this case, females were inseminated without superovulation treatment, does were allowed to breed and after weaning they were superovulated.

Blood samples were collected six days after each superovulation treatment was started (6, 36, 69 and 159 days from the first superovulation treatment, for the first, second, third and fourth superovulation treatment, respectively).

Ovulation rate was checked by laparoscopy after blood sampling.

Detection of anti-FSH Antibodies

Indirect ELISA was used to detect anti-FSH antibodies with an adapted protocol based on Haller *et al.* (2007). Briefly, polystyrene 96-weel plates (Corning) were coated with 1.1 µg/ml FSH in carbonate-bicarbonate buffer (40 mM Na_2CO_3, 60 mM $NaHCO_3$, pH 9.6) at 100 µl per well. Plates were incubated for 16 h at +4ºC and washed with PBS-T (PBS, 1% Tween 20). Serum samples dilution of 1:50 in blocking buffer (PBS, 0.5% Tween, 5% BSA) were added and incubated for 1.15 h at +37ºC. Horseradish peroxidase-conjugated anti-rabbit immunoglobulin (Ig) were diluted 1:4000 in blocking buffer an added to each well after plates were washed. Plates were incubated for 1.15 h at +37ºC in dark. Orthophenylamine and H_2O_2 in developing buffer (Citric acid, Na_2HPO_4) were added after washing plates and colour developing was blocked after 10 min.

Statistical analyses

The effect of repeated superovulation treatment on the ovulation rate and anti-FSH antibodies formation was analyzed by a General Linear Model (Statgraphics®Plus5.1, Statistical Graphics Corp., Rockville, MO, USA).

RESULTS AND DISCUSSION

The ovulation rate was significantly higher in does treated with rhFSH than in control group. In superovulation group, ovulation rate was significantly higher in does treated for the first time with rhFSH than in those treated two, three or four times ($P<0.05$, Table 1). Similar results were obtained in rabbits by Mehaisen *et al.* (2006) with oFSH and eCG who reported that stimulated females presented a significantly higher ovulation rate in the first treatment than in the second. In other species, such as cows (Lubbadeh *et al.*, 1980), cats (Swanson *et al.*, 1996), and hamster (Spanel-Borowski., 1996) a decrease in ovarian response or ovulation rate was detected when the superovulatory treatment was repeated. In contrast, no differences in the number of recovered oocytes, prolificacy or fertility after repeated treatments were found in cows (Lubbadeh *et al.*, 1980), ewes (Roy *et al.*, 1999), women (Caligara *et al.*, 2001), and wallaby (Magarey *et al.*, 2003).

Table 1: Effect of repeated rhFSH superovulation treatments (S1-S4) on ovulation rate and anti-FSH antibody production. Data represent least square means and standard errors; in parenthesis number of cases

	Control	S1	S2	S3	S4	Total
Ovulation rate	8.7 ± 1.42^{c} (22)	19.3 ± 1.36^{a} (24)	13.5 ± 1.26^{b} (28)	13.0 ± 1.28^{b} (27)	14.3 ± 1.31^{b} (26)	13.77 ± 0.59 (127)
Anti-FSH response	0.16 ± 0.073^{b} (20)	0.14 ± 0.082^{b} (16)	0.15 ± 0.091^{b} (13)	0.46 ± 0.079^{a} (17)	0.50 ± 0.077^{a} (18)	0.28 ± 0.036 (84)

Values within the same row with different letter differ significantly ($P<0.05$)

On the other hand, our results indicate that there was a significant difference in the immune response of does after the first two rhFSH superovulatory treatments versus does after the third and fourth treatments ($P<0.05$, Table 1). Whereas, none of the treated females presented an immune response in both the first and second treatments, on the contrary, 40 to 60% of females presented high antibody level following the third and forth treatments. In rabbits, an increase in anti-eCG antibodies has been found after repeated treatments with low doses of eCG (25-40 IU) (Bourdillon *et al.*, 1992; Boiti *et al.*, 1995; Lebas *et al.*, 1996). After seven eCG treatments, 30% of treated does developed an immune response which increased in proportion to the number of injections received (Lebas *et al.*, 1996). These results agree with ours, where females which responded after the third treatment maintained the immunological competence also after the last one. Swanson *et al.* (1996) found an increase in anti-eCG titer immediately after the superovulation treatment in cats. In sheep and wallaby (Roy *et al.*, 1999; Magarey *et al.*, 2003), the highest immune response appeared few days after the injection; but Roy *et al.* (1999) reported that the response appeared earlier after the second treatment than after the first one. On the other hand, Mehaisen *et al.* (2006) observed a decrease in ovulation rate after the second treatment with eCG or FSH, but when the hormones were exchanged, the normal ovulation rate was restored, suggesting that this effect could be related to the immune response. In our study, blood was collected immediately after rhFSH injection. We appreciated that, while ovulation rate and superovulation response diminished in the second treatment, the specific immune response was detected in the third one. It would be possible that in the second treatment there were anti-FSH antibodies but their production began few days later.

In relation to the immunological effect on reproductive response, in the present experiment, the subsequent fertility after artificial insemination (AI) of rabbit does superovulated three times was not significantly different whatever the anti-FSH antibody titer level (data not shown). To date, the effect of anti-eCG production on reproduction of rabbits has been controversial; some authors appreciated that those rabbits which had no immune response, had higher good quality embryos (Boiti *et al.*,1995),

while others did not found any effect of anti-eCG antibodies on receptivity, fertility or prolificacy (Lebas *et al.*, 1996). In human it has been reported that women with infertility problems had a higher anti-FSH antibodies than healthy women (Haller *et al.*, 2005; Haller *et al.*, 2007).

A decrease in reproductive response and in embryo quality has been related to the interval among treatments in different species (Lubbadeh *et al.*, 1980; Swanson *et al.*, 1996; Kanayama and Osada, 2000; Van Blerkom and Davis, 2001); however in some studies reproductive response was not affected by it (Caligara *et al.*, 2001). Our findings indicated that, after extending the time between treatments more than one month, neither the ovulation rate nor superovulation response were enhanced. Moreover, the number of females which presented sera anti-FSH antibodies increased.

CONCLUSIONS

The results of the present study clearly demonstrate that repeated rhFSH superovulation treatments in rabbit does induce a immune response and have a negative effect on ovulation rate. Although anti-FSH antibodies induce a decrease in superovulation response, the ovulation rate of females superovulated two, three, and four times was significanty higher than control females. The subsequent fertility after AI in rabbit does superovulated three times was not affected by the anti-FSH antibody level. The immune response developed shows an important individual variability and may be related with the decreased reproductive response following repeated treatments. Nevertheless, since there were females in which ovulation rate diminished without an increase in sera antibodies; it is clear that reproduction failure after consecutive superovulation treatments can be caused by different reasons, which have to be studied in future.

REFERENCES

Baselga M. 2004: Genetic improvement of meat rabbits. Programmes and diffusion. *In: Proc. 8th World Rabbit Congress, 2004 September, Puebla, Mexico, 14-25.*

Besenfelder U., Haas C., Brem G. 2000. Reproduction technology and gene transfer in rabbits. *In: Proc. 7th World Rabbit, 2000 July, Valencia, Spain, Vol. A, 37-59.*

Boiti C., Castellini C., Canali C., Zampini D., Monaci M. 1995. Long term effect of PMSG on rabbit does reproductive performance. *World Rabbit Sci., 3, 51-56.*

Bourdillon A., Chmitelin F., Jarrin D., Parez V., Rouillere H. 1992. Effects of a PMSG treatment on breeding results of artificially inseminated rabbits. *J. Appl. Rabbit Res., 15, 530-537.*

Caligara C., Navarro J., Vargas G., Simón C., Pellicer A., Remohí J. 2001. The effect of repeated controlled ovarian stimulation in donors. *Hum. Reprod.. 16, 11, 2320-2323.*

Chrenek P., Makarevich A., Vasicek D., Laurincik J., Bulla J., Gajarská T., Rafay J. 1998. Effects of superovulation, culture and microinjection on development of rabbit embryos in vitro. *Theriogenology, 50, 659-666.*

Combelles C.M.H., Albertini D.F. 2003. Assessment of oocytes quality following repeated gonadotropin stimulation in the mouse. *Biol. Reprod., 68, 812-821.*

Forcada F., López M. 2000. Repeated surgical embryo recovery and embryo production in rabbits. *Anim. Repr. Sci., 64, 121-126.*

García M.L., Baselga M. 2002. Estimation of correlated response on growth traits to selection in litter size of rabbits using a cryoopreserved control population and genetic trends. *Livest. Prod. Sci., 78, 91-98.*

Gondret F., Larzul C., Combes S., Rochembeau H. de 2005. Carcass composition, bone mechanichal properties, and meat quality traits in relation to growth rate in rabbits. *J. Anim. Sci., 83, 1526-1535.*

Haller K., Mathieu C., Rull K., Matt K., Bene M.C., Uibo R. 2005. IgG, IgA and IgM Antibodies against FSH: serological markers of pathogenic autoimmunity or of normal immunoregulation? *Am. J. Reprod. Immunol., 54, 262-269.*

Haller K., Salumets A., Grigorova M., Talja I., Salur L., Béne M.C. 2007. Putative predictors of antibodies against follicle-stimulating hormone in female fertility: a study based on in vitro fertilization patients. *Am. J. Reprod. Immunol., 57, 193-200.*

Kanayama K., Osada H. 2000. The yield of abnormal unfertilized eggs observed after repeated gonadotrophin-induced ovulation. *J. Int. Med. Red., 28, 24-27.*

Kauffman R.D., Schmidt P.M., Rall W.F., Hoeg J.M. 1998. Superovulation of rabbits with FSH alters in vivo development of vitrified morulae. *Theriogenology, 50, 1081-1092.*

Lebas F., Theau-Clement M., Remy B., Drion P., Beckers J.F. 1996. production of Anti-PMSG Antibodies and its relation to the productivity of rabbit does. *World Rabbit Sci., 4, 57-62.*

Löseke A., Spanel-Borowski K. 1996. Simple or repeated induction of superovulation: a study on ovulation rates and microvessel corrosion casts in ovaries golden hamsters. *Anna Anat., 178, 5-14.*

Lubbadeh W.F., Graves C.N., Spahr S.L. 1980. Effect of repeated superovulation on ovulatory response of dairy cows. *J. Anim. Sci., 50(1).*

Magarey G.M., Rodger J.C., Buist J.M., Mate K.E. 2003. Effects of repeated superovulation and surgical oocyte collection on ovarian response and natural breeding ability of the tammar wallaby (*Macropus eugenii). Reproduction, 125, 701-707.*

Medjdoub N., Forcada F., López M. 2000. Embryo production from repeated induction of ovulation in gigante de España does. *In: Proc. 7th World Rabbit Congress, 2000 July, Valencia, Spain, Vol A., 191-195.*

Mehaisen G.M.K., Vicente J.S., Lavara R. 2004. In vivo embryo recovery rate by laparoscopic technique from rabbit does selected by growth rate. *Reprod. Dom. Anim., 39, 347-351.*

Mehaisen G.M.K., Vicente J.S., Lavara R., Viudes de Castro M.P. 2005. Effect of eCG dose and ovulation induction treatments on embryo recovery and in vitro development post-vitrification in two selected lines of rabbit does. *Ani. Repro. Sci., 90, 175-148.*

Mehaisen G.M.K., Viudes de Castro M.P., Vicente J.S., Lavara J. 2006. In vitro and in vivo viability of vitrified and non-vitrified embryos derived from eCG and FSH treatment in rabbit does. *Theriogenology, 65, 1279-1291.*

Roy F., Combes B., Vaiman D., Cribiu E.P., Pobel T., Deletang F., Combarnous Y., Guillou F., Maurel M.C. 1999. Humoral immune response to Equine Gonadotropin in ewes: association with majot histocompatibility complex and interference with subsequent fertility. . *Biol. Reprod., 61, 209-218.*

Salvetti P., Theau-Clément M., Beckers J.F., Hurtaud J., Guérin P., Neto V., Falières J., Joly T. 2007. Effect of the luteinizing hormone on embryo production in superovulated rabbit does. *Theriogenology, 67, 1185-1193.*

Swanson W.F., Roth T.L., Graham K., Horohov D.W., Godke R.A. 1996. Kinetics of the humoral immune response to multiple treatments with exogenous gonadotropins and relation to ovarian responsiveness in domestic cats. *Am J Vet Res., 57, 302-307.*

Van Blerkom J., Davis P. 2001. Differential effects of repeated ovarian stimulation on cytoplasmatic and spindle organization metaphase II mouse oocytes matured in vivo and in vitro. *Hum. Reprod., 16(4), 757-764.*

Vicente J.S., Viudes de Castro M.P., García M.L., Baselga M. 2003. Effect of rabbit line on a program of cryopreserved embryos by vitrification. *Reprod. Nutr. Dev., 43, 137-143.*