Indian Journal of Animal Sciences **92** (9): 1056–1060, September 2022/Article
https://doi.org/10.56093/ijans.v92i9.123917

Super-ovulatory response of pregnant mare serum gonadotropin (PMSG) treatment on ovarian characteristics of rabbit does under heat stress

RAOUIA BEN RHOUMA[1✉], MOHAMED AROUA[2] and NACEUR M'HAMDI[3]

Agricultural Higher School of Mograne, Carthage University, 1121 Mograne, Zaghouan, Tunisia

Received: 14 May 2022; Accepted: 28 July 2022

ABSTRACT

The main aim of this study was to determine the effects of superovulation treatment on ovarian characteristics, follicle and embryo number of rabbit does under heat stress. Fourty-eight hours before natural mating, 30 rabbits were divided into three equal groups, viz. control (G1) were injected with 0.1 ml saline/doe and two treatment groups (G2, G3) were injected with 20 IU and 40 IU of pregnant mare serum Gonadotropin (PMSG). After 72 h of natural mating, does in all groups were slaughtered. The genital tract measurement was taken, the embryos were morphologically evaluated, and oocytes and follicle count were recorded. No significant effect of PMSG on ovarian measurement was observed. For corpora lutea number and embryo number from the oviduct and uterine horn, there were no significant effects of PMSG in all the groups.

Keywords: Corpora lutea, Embryo, PMSG, Rabbit, Superovulation

Heat stress is considered as one of the main factors affecting the oxidative stress that leads to an impaired physiological and reproductive feature in rabbit does (El-Ratel *et al*. 2020).

Higher ambient temperature (AT) negatively affects metabolism, energy, water, mineral, and hormonal balances of rabbits (Ondruska *et al*. 2011). Reproductive hormones in females were found to be affected by higher AT, in terms of decreasing levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (Takahashi 2012). Higher AT negatively affected the stages of growth and maturation of follicles and oocytes (Boni 2019). These effects inhibit ovarian follicular development and ovulation rate (Arabameri *et al*. 2017). On commercial farms, rabbit productivity is subjected to negative impacts, leading to reduced longevity. Thus, there is a need to improve the productive efficiency of the rabbit under high AT, through the development of new reproductive strategies to induce doe ovulation (Munari *et al*. 2019).

The use of hormonal treatments for estrous induction and synchronization is a common practice in industrial rabbit breeding. Pregnant mare serum gonadotropin, a very potent compound, obtained from the serum of pregnant mares is the most widely used hormone for lactating does (Castalini *et al*. 1996). A clear positive effect of PMSG was found to be limited to the first 4 cycles of insemination in lactating does (Theau-Clément *et al*. 1996), although a dose-response effect may be involved in this process. PMSG doses commonly used in commercial rabbit farms (20–25 IU) probably exceed the minimal dose needed for improving productivity in lactating does (Theau-Clément et *al*. 1998a), and the lower the PMSG dose and the number of injections, the minor the immune response (Theau-Clément *et al*. 1998b).

Our study aimed to examine the influence of pregnant mare serum gonadotropin (PMSG) on the fertility of rabbits, whose ovarian cycle and ovulation were induced by gonadotropins. The numbers of ovarian corpora lutea, ovulated oocytes and oocyte-derived embryos reaching the blastocyst stage were determined.

MATERIALS AND METHODS

Animals: Lactating multiparous rabbits (30) belonging to the National Agronomic Institute of Tunisia (INAT) strain, obtained after several crossing between the Fauve de Bourgogne, New Zealand and local breeds, at age of 6±1 month and live weight of 3740±210.65 g, were selected. Rabbit does were housed in individual cages and were fed *ad lib.* with commercial balanced feed with 18% crude protein and 12% crude fibre.

During the experimental period, average of AT and relative humidity (RH) were recorded around the cages using a digital thermometer and hygrometer. Thermo-humidity index (THI) value was calculated according to the formula of Marai *et al*. (2001).

Present address: [1]Laboratory of Agricultural Production Systems and Sustainable Development (SPADD), LR03AGR02, Agricultural Higher School of Mograne, Carthage University, 1121 Mograne, Zaghouan, Tunisia. [2]Higher School of Agriculture of Kef, Jendouba University, University Complex Boulifa el Kef 7100. [3]Research Laboratory of Ecosystem and Aquatic Resources, National Agronomic Institute of Tunisia, Carthage University, 43 Avenue Charles Nicolle, Tunis 1082, Tunisia. ✉Corresponding author email: benrhouma18@mail.com

$$THI = db\ °C - [(0.31 - 0.31 \times RH) \times (db\ °C - 14.4)]$$

where, db °C, dry bulb temperature. The values obtained indicate: < 27.8 = absence of heat stress; 27.8 – 28.8 = moderate heat stress; 28.9 – 29.9 = severe heat stress; while > 30.0 signified very severe heat stress.

Protocols of superovulation: Thirty rabbits were divided in three groups, viz. control (G1) was injected with 0.1 ml saline/doe and two treatment groups (G2, G3) were injected with 20 IU and 40 IU of PMSG respectively. The rabbits were administered PMSG injection 48 h before natural mating.

Morphology of the ovaries: The rabbits were slaughtered 72 h after natural mating. Ovarian measurements including weight and number of corpora lutea were recorded on the left and right ovary of each doe.

Follicle number of rabbit does: After slaughtering, 2 ovaries from each rabbit were fixed in Bouin solution followed by processing in paraffin, sectioning, and staining with hematoxylin and eosin. After staining, the follicles were categorized as primordial, primary, secondary, mature or atretic (Figs 1 and 2).

The total number of antral follicles and atretic follicles per ovary was determined by taking an average of the counts from 3 sections (5 sections apart) cut along the long axis of the entire ovary. Also, for each follicular stage, the follicle and atretic follicles were counted with Apotome Fluorescence Microscope (Axio vision Rel 4.8), by taking the diameters of the follicles and that of the oocytes.

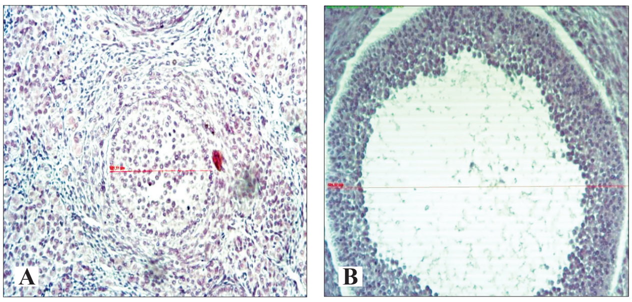

Fig. 1. Histological sections of rabbit ovarian antral non-ovulated follicles. Staining with eosin-haematoxylin. Magnification 300×. A: Atretic follicle; B: First stages of atresia (control group). Interstitial cells (IC) are intact, some granulose cells contain pyknotic nuclei, some granulosa cells are present in follicular fluid (arrow).

Embryos production: The embryos were recovered after slaughtering at 72 h post-natural mating from both superovulated (Groups 2 and 3) and control (Group 1) does. At 72 h post-natural mating, embryos were at the compact morula stage. The genital tracts were immediately removed and the embryos were recovered into a petridish (5 cm diameter) by normo-grade perfusion of each oviduct and the uterine horns. The embryos were counted and evaluated morphologically under a binocular microscope (G ×10). Ovaries were observed and the number of pre-ovulatory follicles were counted.

Statistical analyses: The results obtained for each of the studied variables were subjected to a simple analysis of variance, to prove the existence of significant differences between the three groups with different doses of PMSG in the macroscopic variables (length of right and left ovaries, length of right and left uterine horns, cervix and vagina length) and microscopic variables (primordial, primary, secondary and tertiary follicles).

Data were analyzed using the GLM procedure of SAS software version 9.4 (SAS 2012). Differences between the means values were detected using the SNK test. The results are expressed as mean±standard deviation (SD).

RESULTS AND DISCUSSION

Climatic conditions: In the current study, average ambient temperature and humidity-temperature index were 30.38±1.49°C and 28.65±1.00, respectively. These results indicated that rabbit does suffered from moderate heat stress throughout the experimental period, as reflected by high average values of THI (Marai *et al*. 2002). The change from moderate to severe heat stress affected the production and the reproduction of rabbit does (Ogunjimi *et al*. 2008).

Effect of superovulation treatment on the ovarian morphology: The effect of superovulation treatment on the morphological features of the reproductive organs is given in Table 1. The superovulation treatment did not significantly affect weight of ovary among relative values for G1, G2 and G3. No significant difference among relative values of genital segments (oviduct and uterine horns) from different experimental groups was detected.

PMSG administration imparted a non-significant ($P>0.05$) effect to ovarian weight. This result is in agreement with several authors showing that ovarian

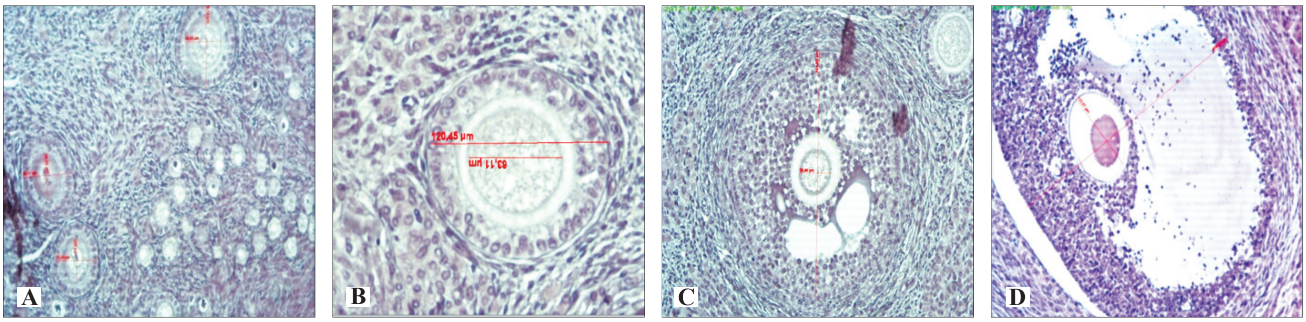

Fig. 2. Histological sections of rabbit ovarian antral non-ovulated follicles. Staining with eosin-haematoxylin. Magnification 300×. A: Primordial and primary follicles with intact granulosa, and theca externa and theca interna cells (arrow); B: Secondary follicles; C: Tertiary follicles; D: Graafian follicle.

Table 1. Morphological analysis of the reproductive organs

Parameter	G1	G2	G3	P
Weight of genitals (g)	19.75±1.15	16.25±1.09	18.81±0.95	0.08
Weight of right ovary (g)	0.35±0.04	0.31±0.03	0.27±0.03	0.31
Weight of left ovary (g)	0.32±0.04	0.32±0.03	0.31±0.03	0.94
Length of right ovary (cm)	1.67±0.09	1.67±0.09	1.79±0.08	0.52
Length of left ovary (cm)	1.66±0.08	1.56±0.08	1.78±0.07	0.13
Width of right ovary (cm)	0.55±0.05	0.60±0.05	0.65±0.04	0.39
Width of left ovary (cm)	0.57±0.05	0.62±0.05	0.64±0.04	0.58
Length of right oviduct (cm)	17.78±0.72	16.42±0.67	16.31±0.57	0.25
Length of left oviduct (cm)	15.50±0.55	14.10±0.51	14.87±0.43	0.19
Length of right uterine horn (cm)	9.27±1.21	10.51±1.08	10.55±0.95	0.67
Length of left uterine horn (cm)	9.10±1.14	9.90±1.02	10.60±0.89	0.56

weight of doe rabbits was not affected by treatment with 100-150 IU PMSG (Kennelly and Foot 1965), 0.5 mg FSH (Yuonglai 1984) or PMSG and hCG (Daader *et al*. 2003).

On the contrary, Fukunari *et al.* (1990) found that the ovarian weight of immature Japanese White rabbit does (4 months old) treated with PMSG (50 IU) was significantly higher than that of the untreated does after 72 h of mating. Also, Gosalves *et al.* (1994) noted that females treated with PMSG had heavier ovaries (0.60 g) than those of does injected with the saline solution (0.18 g).

Effect of superovulation on the corpus luteum and embryos number: The number of rabbit showing CLs on the right ovaries was higher than those showing CLs on the left ovaries in G1 (4.88±0.96 vs. 3.22±0.64), being the opposite in G2 (3.70±0.91 vs 3.20±0.61) and similar in G3 (5.15±0.80 vs 3.76±0.53).

The overall mean number of embryos on right and left oviducts and corpus luteum was 8.43±2.84 for control group, 7.00±2.66 for G2 (20I U of PMSG) and 11.08±2.17 for G3 (40 IU of PMSG) (Table 2).

The number of embryos recorded immediately after slaughter were not affected significantly (P>0.05) by superovulation protocols (Table 2).

The mean number of corpus luteum and embryos did not differ significantly (P>0.05).

Our finding are in disagreement with those of Fahim (2008) who found higher number in NZW does superovulated with PMSG (150 IU) followed by hCG (75 IU) 72 h later and slaughtered 24 or 72 h after mating. In agreement with the present results, Ömer *et al.* (2002) recorded that number of corpora lutea was greater in GnRH treated mice than in control. Similar trend was observed by El-Keraby *et al.* (1991), who found that increasing GnRH dose from 0.2 to 0.4 ml/doe increased number of CL in NZW doe. Generally, number of corpora lutea ranged between 7.4 to 10.3/doe (El-Keraby *et al.* 1991) or averaged 22/doe (Dorra *et al*. 2013) in different rabbit breeds treated with GnRH as compared to 6.6-8.0 or 15.58/doe in the control, respectively. Moreover, Mehaisen *et al.* (2005) recorded greater number of ovulation sites (15.3 and 15.9) as compared to 13.5 and 13.2 (Vicente *et al.* 2003) for doe rabbits of R and V lines, respectively. On the contrary, higher number of corpora lutea (19.2/doe) for rabbits superovulated with PMSG was reported by Lee *et al.* (1991). Salvetti *et al.* (2007) reported that the total number of the corpus luteum was 11.9±3.1 in control, 25.4±8.7 in FSH group without LH and 25.9±10.5 in the FSH group with LH. The results from the present study demonstrate that the total number of embryos was not improved by ovarian stimulation using PMSG.

On the contrary, Salvetti *et al*. (2007) found that during hormonal treatment with FSH, the number of embryos was 19.9±9.3. Also, Theau-Clement *et al*. (2008) recorded a significant increase (7.7 vs 10.9 and 10) in the total number of embryos for the control, 8 and 25 IU eCG, respectively.

In accordance with the present results, Dorra *et al*. (2013) found no difference in embryo recovery rate of control rabbit does and those superovulated with GnRH, but reported higher recovery rate, being 82.87 and 82.24, respectively. However, Fahim (2008) found that number of embryos collected after 72 h of mating was 4.35/ovary and recovery rate was 66.7% in NZW rabbits superovulated by PMSG. Similarly, El-Keraby *et al*. (1991) found that the recovery rates of embryos collected from does treated with

Table 2. Effect of superovulation on the corpus luteum and embryos number

Parameter	G1	G2	G3	P
Number of corpus luteum (right)	4.88±0.96	3.70±0.91	5.15±0.80	0.47
Number of corpus luteum (left)	3.22±0.64	3.20±0.61	3.76±0.53	0.72
Total number of corpus luteum	8.14±2.84	6.90±1.25	8.66±1.14	0.57
Number of embryos of the right oviduct	1.42±0.69	1.75±0.64	2.18±0.55	0.68
Number of embryos of the left oviduct	2.14±0.81	2.00±0.76	2.09±0.65	0.99
Number of embryos of the right corpus luteum	3.16±1.04	1.75±0.90	2.18±0.77	0.59
Number of embryos of the left corpus luteum	1.14±0.60	1.50±0.56	2.00±0.48	0.53
Total number of embryos	8.43±2.84	7.00±2.66	11.08±2.17	0.25

Table 3. Effect of superovulation on the follicle number

Parameter	G1	G2	G3	P
Primordial and primary follicles	235.75±37.48	205.57±44.80	258.93±41.91	0.92
Secondary follicles	7.25±1.17	10.07±1.39	8.31±1.31	0.19
Tertiary follicles	5.40±0.84	7.28±1.01	5.43±0.94	0.33
Graafian follicles	2.55±0.41	3.50±0.50	2.75±0.46	0.13
Number of primordial and primary atretic follicles	5.75±0.98	7.14±1.17	5.37±1.10	0.89
Number of secondary atretic follicles	2.50±0.55	3.35±0.65	2.50±0.61	0.81
Number of tertiary atretic follicles	0.80±0.19	0.64±0.22	0.68±0.21	0.81
Number of atretic follicles mature	0.20±0.14	0.21±0.17	0.37±0.16	0.92

Table 4. Effect of superovulation on the diameter of oocyte and follicle

Variable	G1	G2	G3	P
Diameter of primary oocyte (µm)	14.94±2.49	21.77±2.09	15.50±2.39	0.06
Diameter of primary follicle (µm)	35.25±4.86	45.12±4.35	20.92±3.34	0.00
Diameter of secondary oocyte (µm)	66.03±4.62	63.75±3.58	78.18±4.62	0.05
Diameter of secondary follicle (µm)	144.74±13.41	126.76±10.95	178.85±13.41	0.01
Diameter of tertiary oocyte (µm)	77.86±6.37	77.00±13.52	72.02±6.76	0.81
Diameter of tertiary follicle (µm)	299.79±35.85	308.22±76.05	289.14±48.10	0.97

0.2 or 0.4 ml GnRH ranged from 88.8 to 90% in treated versus 90% in the control group.

Effect of superovulation on the follicle number: The follicle classification was based on the characteristics proposed by Hirshfield and Midgley (1978). PMSG treatment had no effect on number of follicle (Table 3).

The number of primordial, primary, secondary and Graafian follicles were not significantly different between the groups (Table 3). Similar results were reported by El-Nefiawy and Nagwa (2011), who found no significant effects in the primary follicle and antral follicle amounts. However, David *et al*. (2009) noticed the positive effect of ECG on follicular development and ovulation. Currently, there is little information on whether ECG has more remarkable effects on ovarian growth and follicular development than FSH in the same animal. Zhang *et al*. (2007) approved the efficacy of Equine chorionic gonadotropin (ECG, previously known as pregnant mare serum gonadotrophin, PMSG) on the superovulation of mammals due to its efficacies on FSH and LH. Arias-Alvarez *et al.* (2009) found that treatment based on eCG does not affect the number of tertiary and Graafian follicles.

The present number of total follicles/doe was lower than 62.67 follicles/doe as reported by Dorra et *al.* (2013) in Baladi Red rabbits superovulated by PMSG and GnRH, and higher than 30.4/doe in Baladi Black and 20.8/doe in NZW does superovulated by GnRH as reported by Abdel-Khalek *et al.* (2010).

The mean number of primordial and primary atretic follicles was 5.75±0.98, 7.14±1.17 and 5.37±1.10, respectively for the control, G2 and G3 groups. The mean number of secondary atretic follicles was 2.50±0.55 for G1, 3.35±0.65 for G2 and 2.50±0.61 for G3. The average number of tertiary atretic follicles was 0.80±0.19, 0.64±0.22 and 0.68±0.21 respectively for the control group, G2 and G3 groups. The mean number of atretic Graafian follicles was 0.20±0.14, 0.21±0.17 and 0.37±0.16 respectively for the control group, G2 and G3.

Zhang *et al*. (2007) found that FSH inhibits the granulosa cell apoptosis and can rescue granulosa cells from atresia in mice. Garcia *et al.* (2009) demonstrated that treatment with eCG had no effect on the rate of atretic follicles with both intensive and semi-intensive rates.

Effect of superovulation on the diameter of oocyte and follicle: Significant effect was observed on diameter of oocyte and primary follicle in G2 group with 20 IU of PMSG dose and secondary with 40 IU of PMSG dose (Table 4). However, the diameter of tertiary oocyte as well as tertiary follicle was not affected by PMSG injection.

It was concluded that superovulation treatment of rabbit does with 20 and 40 IU PMSG, after natural mating, had no significant effect on ovarian weight, embryo yield and follicle number.

REFERENCES

Abdel-Khalek A E, Ganah H A B and Shehab El-Din A M. 2010. Effect of PMSG administration in relation to follicular diameter on superovulatory response and embryo quality in Friesian cows. *Journal of Animal and Poultry Production* **1**(1): 23–32.

Arabameri A, Sameni H and Bandegi A. 2017. The effects of propolis extract on ovarian tissue and oxidative stress in rats with maternal separation stress. *International Journal of Reproductive Biomedicine* **8**: 509–20.

Arias-Alvarez M, Garcı´a-Garcı´a R M, Rebollar P G, Nicodemus N, Revuelta L, Milla´n P and Lorenzo P L. 2009. Effects of a lignin-rich fibre diet on productive, reproductive and endocrine parameters in nulliparous rabbit does. *Livestock Science* **121**: 227–33

Boni R. 2019. Heat stress, a serious threat to reproductive function in animals and humans. *Molecular Reproduction and Development* **86**: 1307–32.

Castellini C. 1996. Recent advances in rabbit artificial insemination. *Proceedings of the 6th World Rabbit Congress, Toulouse* **2**: 13–26.

Daader A H, El-Darawany A A, Zeidan A A and Sheiha A M.

2003. Ovarian activities of rabbit does as affected by season and PMSG and HCG treatments. *Egyptian Journal of Rabbit Science* **13**(1): 75–88.

David E N, Timothy J P and Gary C W E. 2009. *Veterinary Reproduction and Obstetrics*. 9th Edn., Philadelphia, USA, Elsevier Medicine Press. Pp. 127-189.

Dorra Tork M, Kh. E L Sherif, M N Shamiah and Sara F Fouda. 2013. Effect of superovulation on ovulatory response, embryo recovery and embryo measurements in Baladi Red Rabbit does. *Journal of. Animal and Poultry Production, Mansoura University* **4**(7): 429–40.

El-Keraby F, Tork M Dorra, Abd El-Ghani A I and Fawzy S A. 1991. Effect of GnRH administration rate and embryo recovery in some rabbit breeds. *Journal of Agriculture Sciences Mansoura University* **16**(8): 1749.

El-Nefiawy N. 2011. Effect of exogenous prostaglandin E2 administration on ovarian follicle growth and angiogenesis in rat with reference to pregnant mare serum gonadotrophin. *Egyptian Journal of Histology* **34**: 251–59.

El-Ratel I T, Abdel-Khalek A E, Gabr S A, Hammad M E and El-Morsy H I. 2020. Influence of allicin administration on reproductive efficiency, immunity and lipid peroxidation of rabbit does under high ambient temperature. *Journal of Animal Physiology and Animal Nutrition* **104**: 539–48.

Fahim H N. 2008. 'Some physiological and nutritional studies on rabbits.' Ph.D. Thesis, Poultry Production Department, Mansoura University.

Fukunari A, Maeda T, Terada T and Tsutsumi Y. 1990. The recovery of follicular oocytes from the rabbit ovary during prepubertal age and *in vitro* maturation of their oocytes. *Japanese Journal of Animal Reproduction* **36**: 1–8.

Gosalves L E, Alvarino J M R and Diaz P T M. 1994. Influence of age, stimulation by PMSG or flushing on the ovarian response to LHRHa in young rabbit females. *World Rabbit Science* **2**: 41–45.

Hirshfield A N and Midgley A R Jr. 1978. Morphometric analysis of follicular development in the rat. *Biology of Reproduction* **19**: 597–605.

Lee T S, Lim K T and Na J S. 1991. Superovulation treatment and short term storage of embryos in domestic rabbit. *Korean Journal of Animal Science* **33**: 18–24.

Marai I F M, Ayyat M S and Abd El-Monem U M. 2001. Growth performance and reproductive traits at first parity of New Zealand white female rabbits as affected by heat stress and its alleviation under Egyptian conditions. *Tropical Animal Health and Production* **6**: 451–62.

Marai I F M, Habeeb A A M and Gad A E. 2002. Rabbits, productive, reproductive and physiological performance traits as affected by heat stress: A review. *Livestock Production*.

Munari C, Ponzio P, Alkhawagah A R, Schiavone A and Mugnai C. 2019. Effects of an intravaginal GnRH analogue administration on rabbit reproductive parameters and welfare. *Theriogenology* **125**: 122–28.

Ogunjimi L A O, Oseni S O and Lasisi F. 2008. *Influence of temperature humidity interaction on heat and moisture production in rabbit*. 9th World Rabbit Congress, Verona, Italy.

Ondruska L, Rafay J, Okab A B, Ayoub M A, Al-Haidary A A, Samara E M, Parkanyi V, Chrastinova L, Jurcik R, Massanyi P, Lukac N and Supuka P. 2011. Influence of elevated ambient temperature upon some physiological measurements of New Zealand White rabbits. *Veterinarni Medicina* **56**: 180–86.

Salvetti P, Guerin P, Theau-Clement M, Hurtaud J, Bechers J F and Joly T. 2007. Essais d'amélioration de la production d'embryons chez la lapine, *12ème journée de la recherché cunicole*, Mans France, 41-44 p.

SAS. 2012. Statistical Analysis System, release 9.4 2012. SAS Institute Inc., Cary, NC, USA.

Takahashi M. 2012. Heat stress on reproductive function and fertility in mammals: Review article. *Reproductive Medicine and Biology* **11**: 37–47.

Theau-Clément M, Lebas F, Boiti C, Brecchia G and Mercier P. 2008. Influence of different eCG doses on sexual receptivity and productivity of rabbit does. *World Rabbit Science* **16**: 65–72.

Theau-Clément M and Lebas F. 1996. Effect of a systematic PMSG treatment 48 hours before artificial insemination on the productive performance of rabbit does, *World Rabbit Science* **(4)**: 47–56.

Theau-Clement M, Castellini C, Maertens L and Boiti C. 1998a. Biostimulations applied to rabbit reproduction: Theory and practice. *World Rabbit Science* **6**: 179–84.

Theau-Clément M, Lebas F, Poujardieu B and Mercier P. 1998b. Effect of different doses of PMSG on the induction of sexual receptivity and productivity of does in artificial insemination (Translated). *7èmesJournées de la Recherche Cunicole en France*, Lyon **1998**: 221–23.

Zhang C, Liu L, Di M, Zhao D, Zhao L, Xu P and Rui R. 2007. Effects of the PMSG dosages on superovulation and oocyte quality in immature rats. *Chinese Journal of Comparative Medicine* **17**: 338–44.