

THE EFFECT OF PMSG DOSE ON CORPORA LUTEA AND EMBRYO NUMBERS IN GILTS

Urbánková L., Vejnar J.

Institute of Animal Science Prague, Czech Republic

Abstract

Superovulation in donors is an important factor influencing ovulation rate and embryo recovery. Crossbreed pubertal gilts (n=376) treated from year 2005 to 2009 were used as embryo donors. Experiment was evaluated in two parts: In the first part (I) gilts were divided into two groups with different synchronization schedules. In the first group (R+) 265 gilts were treated with Regumate Porcine (JANSSEN). In the second group (R-) were gilts (n=111) without Regumate diet. The number of ovulations was higher in R+ group (15.1 ± 9.3) compared to R- group (14.9 ± 9.3). There was no significant difference ($p > 0.05$) between both groups. The number of recovered embryos in R+ group (8.75 ± 8.06) and R- group (8.81 ± 6.63) did not significantly differ ($p > 0.05$) between groups.

In the second part of experiment (II) gilts were divided into four groups with different superovulation schedules regardless Regumate treatment. Gilts were synchronized and superovulated by intramuscular injection dose from 400 to 600 i.u. of PMSG - group A (n= 21), dose from 650 to 750 i.u. of PMSG - group B (n= 61), dose from 800 to 850 i.u. PMSG - group C (n= 189) and dose from 900 to 1000 i.u. of PMSG - group D (n= 104) followed 500 i.u. of HCG 72 hours later (both groups). There were significant differences in the number of corpora lutea between groups A-B ($p < 0.05$), groups A-D ($p < 0.05$), groups B-C ($p < 0.05$) and groups C-D ($p < 0.05$). The number of embryos was 9.9 ± 7.4 (group A), 6.1 ± 5.8 (group B), 8.3 ± 6.6 (group C) and 11.3 ± 9.0 (group D). There were significant differences between groups A-B ($p < 0.05$), groups B-C ($p < 0.05$), groups B-D ($p < 0.01$) and groups C-D ($p < 0.01$). The highest number of recovered embryos was in group D which shows a high number of ovulations too.

Key Words: Gilt, PMSG, ovulation, embryo

Synchronization of estrus and superovulation are very important for recovering high quality embryos for experimental purposes. Techniques such as *in vitro* fertilization and embryo manipulation require large numbers of high quality oocytes and embryos. The number of embryos derived from regular cycling sows is not sufficient. Therefore, effective methods to increase the yield of embryos recovered from prepubertal gilts are needed [9].

Such as suitable agent of estrus and ovulation control has been used successfully a peroral application of synthetic progestines (zinkmetallibur, altrenogest, oxolven, trenbolon) for sexual cycle synchronization in pigs [7].

The superovulation is an important factor influencing embryos recovery. It is accepted that a combination of gonadotropins (PMSG and HCG) is used to induce follicular growth and ovulation in prepubertal pigs [3]. Single injection of P.G. 600 containing 400 i.u. of PMSG and 200 i.u. of HCG induced fertile estrus in prepubertal gilts too [1].

The standard treatment to recover high numbers of embryos requires 1000-1500 i.u. PMSG followed by 500-750 i.u. HCG 72 hours later.

Material and Methods

Crossbreed pubertal gilts (n=376) treated during years 2005 to 2009 were used as embryo donors.

Experiment was evaluated in two parts:

In the first part (I) gilts were divided into two groups with different synchronization schedules. In the first group (R+) 265 gilts were treated with Regumate Porcine (JANSSEN) diet for 18 days in dose 5ml (20mg of altrenogest) for synchronization of sexual cycle. In the second group (R-) were gilts (n=111) without Regumate diet. Gilts were treated with different doses of PMSG (Sergon, Bioveta, Ivanovice na Hané) 24 hours after last Regumate treatment (first group) or in follicular phase of cycle (second group). Subsequently gilts (both groups) were treated with 500 i.u. of HCG (Pregnyl, ORGANON or Werfacher, WERFFT AG) 72 hours after PMSG dose.

In the second part (II) gilts were divided into four groups with different superovulation schedules regardless Regumate treatment. Gilts were synchronized and superovulated by intramuscular injection dose from 400 to 600 i.u. of PMSG - group A (n= 21), dose from 650 to 750 i.u. of PMSG - group B (n= 61), dose from 800 to 850 i.u. PMSG - group C (n= 189) and dose from 900 to 1000 i.u. of PMSG - group D (n= 104) followed 500 i.u. of HCG 72 hours later (both groups).

Gilts were inseminated 24 hours after HCG administration. Insemination was replaced twice at 12 hours intervals.

Gilts were slaughtered on the fifth day after initial insemination. The number of ovulated follicles was estimated from the number of corpora lutea present on the surface of both ovaries. Based on the number of ovulated

follicles, the percentage of recovered embryos was calculated (recovery rate).

Embryos were flushed from uterine horns with 40 ml of Dulbecco PBS medium, supplemented with 10% foetal bovine serum (FBS) warmed to 38.5°C. The recovered embryos were all embryos flushed out from uterine horns. These embryos were morphologically examined under stereomicroscope.

Results

The number of ovulations measured by the number of corpora lutea formed on the ovaries and number of obtained embryos were compared between two groups of gilts with (R+) or without (R-) Regumate treatment in the first part of experiment. The number of ovulations was higher in R+ group (15.1 ± 9.3) compared to R- group without Regumate (14.9 ± 9.3). There was no significant difference between both groups. The numbers of recovered embryos in R+ group (8.75 ± 8.06) and R- group (8.81 ± 6.63) did not significantly differ between groups (Fig. 1).

In the second part of experiment, the number of ovulations measured by the number of corpora lutea and number of recovered embryos were compared among four groups of gilts divided by the different PMSG dose

regardless of the Regumate treatment (Fig. 2).

The number of corpora lutea was 12.9 ± 6.1 (group A), 17.0 ± 10.5 (group B), 13.7 ± 7.9 (group C) and 16.8 ± 10.9 (group D). There were significant differences between groups A-B ($p < 0.05$), groups A-D ($p < 0.05$), groups B-C ($p < 0.05$) and groups C-D ($p < 0.05$). That means, the number of ovulations was significantly higher in groups B (650-750 i.u. of PMSG) and D (900-1000 i.u. of PMSG) than in groups A (400-600 i.u. of PMSG) and C (800-850 i.u. of PMSG).

The number of embryos was 9.9 ± 7.4 (group A), 6.1 ± 5.8 (group B), 8.3 ± 6.6 (group C) and 11.3 ± 9.0 (group D). There were significant differences between groups A-B ($p < 0.05$), groups B-C ($p < 0.05$), groups B-D ($p < 0.01$) and groups C-D ($p < 0.01$). The highest number of recovered embryos was in group D (900-1000 i.u. of PMSG) which shows a high number of ovulations too. On the other hand, group B (650-750 i.u. of PMSG) showed the highest number of ovulations but the lowest number of recovered embryos. It relates with percentage of recovered embryos (recovery rate) in particular groups. Recovery rate was 65.8% in group A, 33.2% in group B, 52.5% in group C and 52.0% in group D. There were found out significant differences between groups A-B ($p < 0.001$), A-C ($p < 0.05$), A-D ($p < 0.05$), B-C ($p < 0.001$) and B-D ($p < 0.001$).

Figure 1. Number of Corpora lutea and number of embryos in R+ (with Regumate preparation) and R- (without Regumate preparation) group

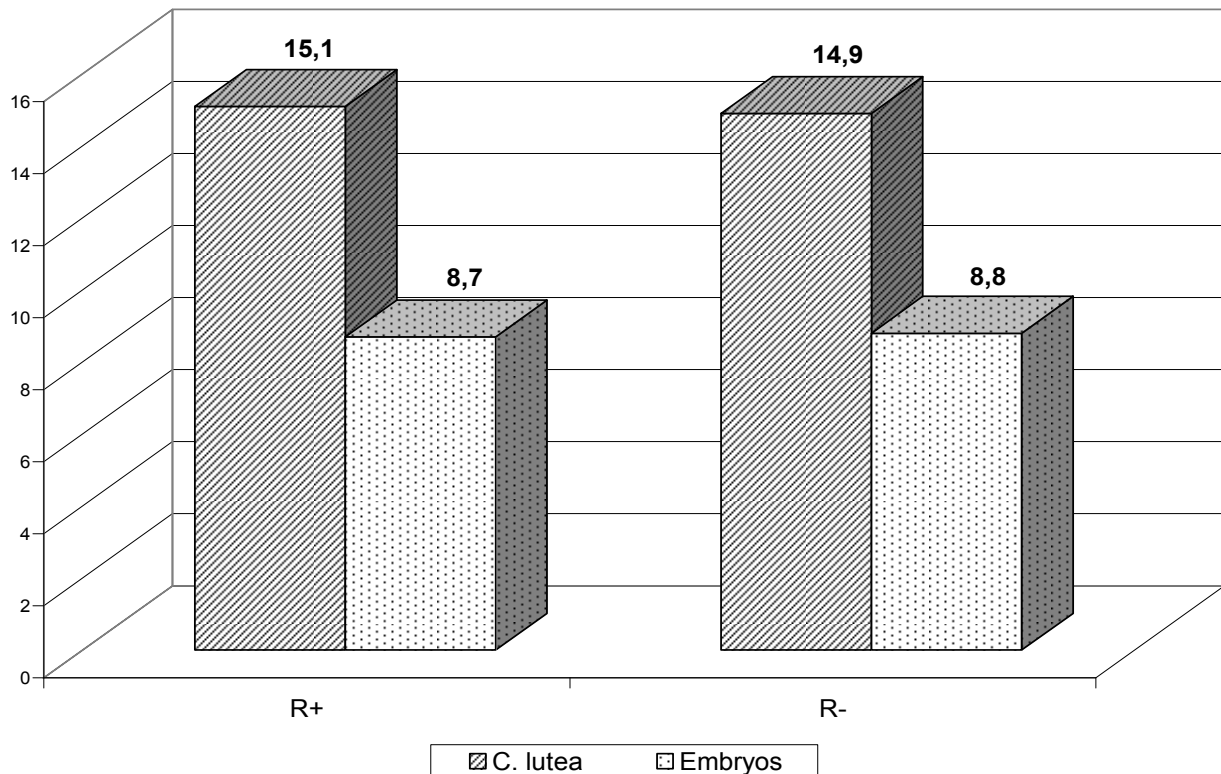
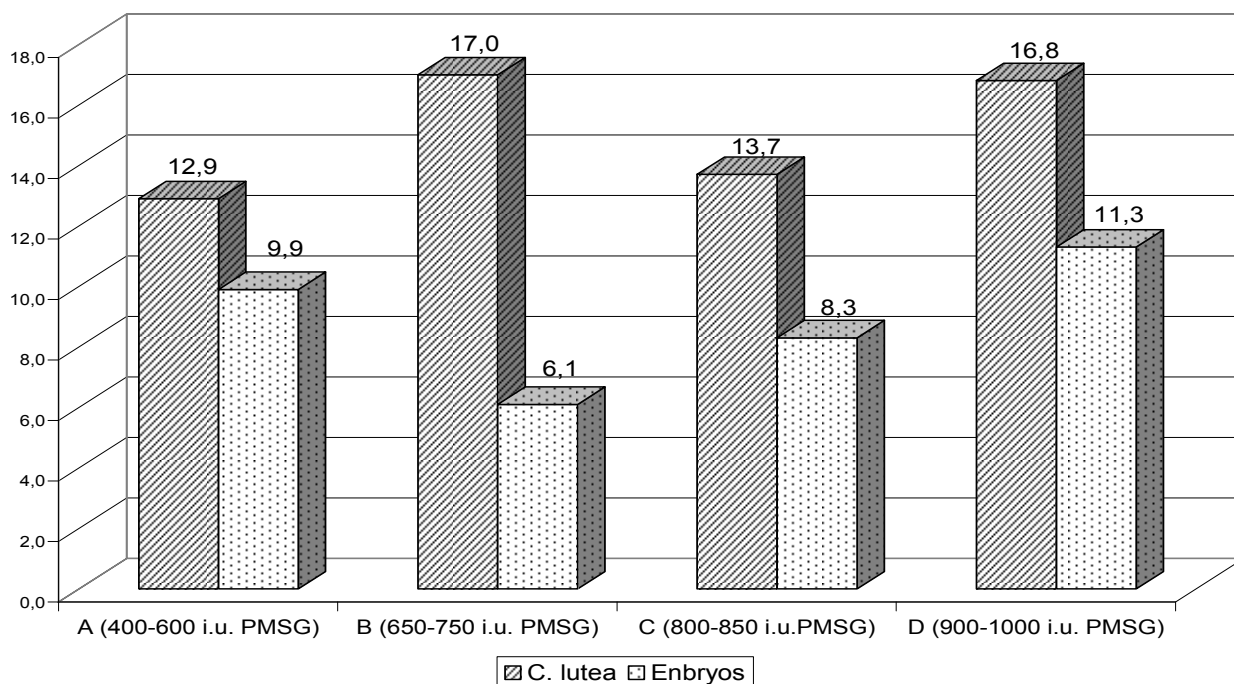


Figure 2. Number of *C. lutea* and numer of embryos in gilts with different PMSG dose

Discussion

In the first part of experiment no significant differences were found out between group of gilts treated and non treated with Regumate. Estienne *et al.* also did not found a beneficial effect of P.G. 600 given after Regumate therapy on the ovarian response [2]. In contrast to our results, pretreatment of prepubertal gilts with progesterone for 8 days prior to injection of 750 i.u. of PMSG improved follicular development and ovarian rate [5]. In summary, the use of Regumate appeared to effectively synchronize estrus in randomly cycling gilts. Regumate administration before PMSG and HCG treatment had no effects on the number of ovulations.

In the second part of experiment, the dose of 900-1000 i.u. of PMSG followed by 500 i.u. of HCG was found as a most optimal. It did the best results with numbers of ovulations with regard to numbers of recovered embryos. Similarly Rátky *et al.* indicated that application of 1000 and 1250 i.u. of PMSG resulted in a higher rate of ovulation compared to 750 i.u. and the number of follicular cysts increased after administration of 1250 i.u. of PMSG compared to 750 and 1000 i.u.. Thus dose of 1000 i.u. of PMSG was chosen as the best [6]. Hazeleger *et al.* found that ovulation rate was lower using 1000 i.u. of PMSG than 1500 i.u. But embryo recovery rate was similar in both doses [4]. Similarly in the study of Řiha *et al.* stimulation of gilts with dose 1000 i.u. of PMSG was comparable with dose 1500 and 2000 i.u. but number of recovered embryos was slightly lower [7]. However, the efficiency of PMSG depends not only on the dosage but also on animal factors, such as age, body weight, and breed [8].

References

- [1] Britt JH, Day BN, Webel SK, Brauer MA. Induction of fertile estrus in prepubertal gilts by treatment with combination of pregnant mare's serum gonadotropin and humane chorionic gonadotropin. *Journal of animal science* 1989; 67:1148-1153.
- [2] Estienne MJ, Harper AF, Horsley BR, Estienne CE, Knight JW. Effects of P.G. 600 on the onset of estrus and ovulation rate in gilts treated with Regu-mate. *Journal of animal science* 2001; 79:2757-2761.
- [3] Guthrie HD, Pursel VG, Wall RJ. Porcine follicle-stimulating hormone treatment of gilts during an altrenogest synchronized follicular phase: effects on follicle growth, hormone secretion, ovulation, and fertilization. *Journal of Animal Science* 1997; 75:3246-3254.
- [4] Hazeleger W, Bouwman EG, Noordhuizen JPTM, Kemp B. Effect of superovulation induction on embryonic development on day 5 and subsequent development and survival after nonsurgical embryo transfer in pigs. *Theriogenology* 2000; 53(5):1063-1070.
- [5] Nephew KE, Cardenas H, Pope WF. Effects of progesterone pre-treatment on fertility of gilts mated at an induced pubertal estrus. *Theriogenology* 1994; 42:99-106.

- [6]Rátky J, Brússow KP, Solti L, Torner H, Sarlós P. Ovarien response, embryo recovery and results of embryo transfer in a Hungarian native pig breed. *Theriogenology* 2001; 56(5):969-78.
- [7]Říha J, Vejnar J, Čunát L. The effect of PMSG dose on embryo number and quality in sows and gilts. *Czech journal of animal science* 1997; 42(5):213-218
- [8]Smorag Z, Gajda B, Jura J, Skrzyszowska M, Pasięka J. Factors affecting the production of oocytes in superovulated pigs: seven-year retrospective studies. *Annals of Animal Science* 1999; 26(4):155-161.
- [9]Zięcik AJ, Białłowicz M, Kaczmarek M, Demianowicz W, Rioperez J, Wasielek M, Bogacki M. Influence of estrus synchronization of prepubertal gilts on embryo quality. *Journal of Reproduction and Development* 2005; 51:379-384.

The study was supported by the Ministry of Agriculture of the Czech Republic - project No. QG 50052 and QH 71284