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Influence of Estrus Synchronization of Prepubertal Gilts on Embryo Quality

Adam J. ZIECIK¹⁾, Malgorzata BIALLOWICZ¹⁾, Monika KACZMAREK¹⁾,
Wieslaw DEMIANOWICZ¹⁾, Juan RIOPEREZ²⁾, Marta WASIELAK¹⁾ and
Marek BOGACKI¹⁾

¹⁾*Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, ul. Tuwima 10, 10-747 Olsztyn, Poland* ²⁾*Departamento de Metabolismo y Nutricion, Instituto del Frio, CSIC, Madrid, Spain*

Abstract. Synchronization and superovulation are commonly used to obtain large numbers of embryos for experimental and practical purposes. This study compared the number, quality, and *in vitro* development of embryos recovered from gilts following single or double estrus synchronization and superovulation. Prepubertal gilts from the single synchronization group were injected with 1500 I.U. PMSG and 1000 I.U. hCG 72 h later. The double synchronized group of gilts was treated with 750 I.U. PMSG and 500 I.U. hCG 72 h later. After 17 days, 1500 I.U. PMSG followed by 1000 I.U. hCG was administered. Five days after insemination embryos were recovered and cultured for 6 days. Both single and double hormonal stimulation schedules resulted in recovery of elevated numbers of embryos (28.4 and 23.4 vs. 11.3; $p < 0.01$ and $p \leq 0.05$, respectively) with a higher percentage of embryos classified as degenerated (39.2% and 43.1%, respectively) compared to the non-stimulated, control group (5.1%). The number of embryos destined for culture did not differ between the single and double synchronized groups. The highest percentage of hatched embryos was observed in the control group. In conclusion, the single synchronization and superovulation schedule is sufficient to obtain high numbers of embryos, however, both synchronization methods resulted in the recovery of considerable numbers of degenerated embryos. A higher number and percentage of hatched embryos after culture was found among embryos from the control group compared to gonadotropin-stimulated gilts.

Key words: Embryo quality, Estrus synchronization, Prepubertal gilts

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The introduction of gilts into planned piglet production by batch farrowing requires an efficient method of estrus synchronization. Normally, gilts tend to reach puberty at about 220 days of age if no form of stimulation is provided [1]. Recently, it has become normal to advance puberty in commercial gilts by hormonal stimulation. However, most of the studies that have been done in

the past revealed that stimulation of puberty is fraught with difficulties [2]. Particularly, gilts treated with exogenous gonadotropins show wide variability in pubertal response, fail to exhibit signs of estrus, often fail to undergo a normal estrous cycle following the induced estrus, and exhibit poor subsequent reproductive performance [3, 4], such as small litter size when inseminated in the first synchronized estrus [5].

Synchronization of estrus and superovulation are also 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.

Embryo transfer in the pig is becoming more commonplace in the 21st century as researchers develop new applications of reproductive biotechnology [6]. Efficient embryo transfer is one of the factors influencing the successful production of transgenic pigs [7] for biomedical purposes. Effective methods of controlling the estrous cycle of donor and recipient pigs are required in order to achieve efficient embryo transfer [6].

Effective methods to induce puberty and increase number of good quality embryos have been studied in swine extensively. It is accepted that a combination of gonadotropins (PMSG and hCG) is used to induce follicular growth and ovulation in prepubertal gilts [8].

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. The majority of studies suggest that gilts need to be 150–170 days of age to maximize the pubertal response to boar contact or exogenous hormones [1]. Moreover, Karalus *et al.* [9] indicated that injection of 750–1000 I.U. PMSG and 500 I.U. hCG in 120-day-old gilts consistently results in induction of ovulation but it was not always associated with the estrus behavior.

Hormonal stimulation of the first estrus may elongate the time to the next spontaneous estrus [4, 5]. On the other hand, insemination of gilts in the first induced estrus [10] is very often ineffective. Kapelanski *et al.* [11] confirmed that hormonal synchronization of the first, as well as the second estrus, in a technological group of gilts increases the probability of the fertile second estrus in that group and shortens the period between the first and the second estrus.

Therefore, the purpose of this study was to compare the number, quality, and *in vitro* development of embryos obtained from gilts synchronized and superovulated after one or two hormonal treatments.

Material and Methods

Crossbreed prepubertal gilts ($n=17$), 165–180 days old and weighing at least 100 kg were randomly divided into two groups with different synchronization schedules and a control group, and were used as embryo donors. Animals were fed a standard diet (2868 kcal).

Gilts from the first group ($n=10$) were synchronized and superovulated by intramuscular injection of 1500 I.U. PMSG (Folligon, INTERVET) followed by 1000 I.U. hCG (Chorulon, INTERVET) 72 h later. For the first time, gilts were inseminated 24 h after hCG administration. Insemination was repeated twice at 12 h intervals.

The second group of 7 gilts was synchronized two times. Initially, gilts were injected with 750 I.U. PMSG followed by 500 I.U. hCG 72 h later. Subsequently, 17 days after hCG injection, 1500 I.U. PMSG followed by 1000 I.U. hCG after 72 h was administered. Gilts were inseminated 24 h after hCG injection, and this was repeated twice at 12 h intervals.

Control gilts ($n=3$) were inseminated three times at 12 h intervals during their natural estrus.

Gilts from each group 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.

Embryos were flushed from uterine horns with 40 ml 0.9% NaCl, supplemented with 10% newborn calf serum (NCS) warmed to 38.5 °C. The recovered embryos were all embryos flushed out from the uterine horns. These embryos were morphologically examined under stereomicroscope. Embryos classified as morulas and blastocysts were cultured in 500 μ l of NCSU-23 medium (NaCl, KCl, $\text{CaCl}_2 \times 2\text{H}_2\text{O}$, $\text{MgSO}_4 \times 7\text{H}_2\text{O}$, NaHCO_3 , KH_2PO_4 , glucose, glutamine, taurine, hypotaurine, fetal calf serum, penicillin, and streptomycin—Sigma Chemical Co., St. Louis, USA) in a humidified (5% CO_2 ; 38.5 °C) environment and observed daily for 6 days. The number of embryos at different developmental stages and the number of hatched embryos were observed and recorded during culture.

Experiments were conducted according to Local Ethic Committee guidelines and approval. Statistical analysis was conducted with the GraphPad Prism 2.0 software using the unpaired t-test with

Fig. 1. Number and percentage (mean $\pm$ sem) of recovered embryos in the single (white bars) and double hormonally synchronized groups (gray bars) and in the control group of gilts (black bars). Statistical differences are represented by * ($P < 0.05$) and ** ($P < 0.01$).

Fig. 2. Number of embryos (mean $\pm$ sem) recovered from gilts after single (white bars) and double estrus synchronization (gray bars) and from gilts in the control group (black bars) classified as morulas, blastocysts, and degenerated embryos. Statistical differences are represented by * ($P < 0.05$) and ** ($P < 0.01$).

Welch's correction. P values of less than 0.05 were considered to be statistically significant.

Results

The number of ovulations measured by the number of corpora lutea formed on the ovaries was higher after both the single (33.2 ± 4.5 ; $P < 0.05$) and double (29.6 ± 3.1 ; $P < 0.01$) synchronization schedule used in this study when compared to control group (11.3 ± 1.2). There was no difference between the number of ovulations in both synchronized groups.

Similarly, the numbers of recovered embryos in the single and double synchronized groups were higher ($P < 0.01$; $P < 0.05$) compared to the control group. However, the percentage of recovered embryos did not significantly differ between the single and double synchronization groups vs. the control group ($84.2 \pm 3.4\%$ and $80.6 \pm 9.1\%$ vs. $100 \pm 0.0\%$, respectively; Fig. 1).

The number of recovered morulas and blastocysts in both synchronized groups and control group were not significantly different (Fig. 2).

A higher number of embryos classified as degenerated was observed in the single and double hormonally synchronized groups vs. the control group ($P < 0.01$ and $P < 0.05$, respectively; Fig. 2). There was no significant difference in number of degenerated embryos between both synchronized groups.

A higher percentage of recovered morulas was found in the control group compared to both

Fig. 3. Percentage of embryos (mean $\pm$ sem) classified as morulas, blastocysts, and degenerated embryos recovered from gilts after single (white bars) and double (gray bars) estrus synchronization and from gilts in the control group (black bars). Statistical differences are represented by * ($P < 0.05$) and ** ($P < 0.01$).

synchronized groups ($P < 0.05$; Fig. 3). Moreover, the percentage of degenerated embryos in the control group was lower compared to the single and double synchronization group ($P < 0.01$ and $P < 0.05$, respectively). The percentage of blastocysts was not significantly different among the experimental groups.

The number of embryos destined for culture (morulas and blastocysts) in the group with single

Fig. 4. Number of embryos (mean $\pm$ sem) destined for culture (morulas and blastocysts) and number of hatched embryos during incubation, collected from gilts after single (white bars) and double (gray bars) estrus synchronization and from gilts in the control group (black bars). Statistical differences are represented by * ($P < 0.05$).

synchronization tended to be higher compared to the double synchronization group and was higher compared to the control group ($P < 0.05$; Fig. 4). The highest number of hatched embryos was found in the control group. In contrast, the lowest number of hatched embryos was estimated in the group with double synchronization, which was significantly different compared to the control group ($P < 0.05$). The number of hatched embryos in the group with single synchronization was intermediate.

A high percentage of morulas and blastocysts destined for culture was observed in the control group ($94.9 \pm 5.1\%$) compared to the single and double synchronized groups ($60.8 \pm 7.9\%$; $56.9 \pm 15.3\%$), and a statistical difference was observed between both synchronized groups and the control group ($P < 0.05$; Fig. 5). Similarly, a high percentage of hatched embryos was observed in the control group ($78.2 \pm 2.0\%$) compared to both the single and double synchronized groups ($29.5 \pm 7.4\%$; $27.3 \pm 8.8\%$, respectively; $P < 0.01$).

Discussion

In the present study, a higher number of ovulated follicles and embryos were recovered from gilts after using both methods of estrus synchronization and superovulation compared to control gilts. This indicates that single and double synchronization schedules can be applied to obtain satisfactory

Fig. 5. Percentage of embryos (mean $\pm$ sem) destined for culture from the pool of recovered embryos and the percentage of hatched embryos after 6 days of culture, collected from gilts after single (white bars) and double (gray bars) estrus synchronization and from gilts in the control group (black bars). Statistical differences are represented by * ($P < 0.05$) and ** ($P < 0.01$).

numbers of embryos for biotechnological studies.

However after morphological evaluation of the collected embryos, we found a higher percentage of degenerated embryos in both groups of gilts subjected to hormonal treatment. This suggests that exogenous gonadotropins may have an unfavorable effect on oocyte quality and proper development of embryos *in vivo*. The number of morulas and blastocysts destined for culture in the control and double hormonally synchronized groups were similar. Since the highest number of embryos destined for culture was noted in the single synchronized group, better efficiency of this method compared to double synchronization schedule can be suggested.

The number of embryos hatched during the five days of culture obtained from single and double synchronized gilts were similar and lower than in the control group. According to Kapelanski *et al.* [11], hormonal stimulation of the first and the second estrus in prepubertal gilts by PMSG and hCG injection resulted in early and successful mating of gilts and larger litter sizes. These results were obtained when hormonal stimulation was preceded by 25 days of feeding gilts with an insulinogenic diet. In our study, the double synchronization schedule was not more efficient

compared to the single synchronization schedule, but gilts were fed with a standard diet.

In our experiment, the highest number and percentage of hatched embryos were obtained from gilts in the control group. Similarly, in the study of Pinkert *et al.* [12], zygotes obtained from prepubertal gilts after induction of superovulation did not possess the same *in vitro* developmental potential as zygotes from pubertal gilts. Moreover, the authors observed that the development of embryos from prepubertal and mature gilts was similar to the morula but not the blastocyst stage. Wiesak *et al.* [13] showed that the pattern of follicular development in naturally cyclic and PMSG/hCG (750 I.U./500 I.U.) treated gilts varied, and suggested that the ovaries of gonadotropin-treated gilts are functionally different from the ovaries of mature females. This indicates that the highest quality embryos can be collected from gilts during their natural estrous cycle. Unfortunately, for biotechnological purposes, collection of early embryos from larger numbers of gilts during their natural estrous cycle is extremely difficult or impossible.

Smorag *et al.* [7] found that over 95% of gilts responded positively to administered gonadotropins. Similarly, Karalus *et al.* [9] indicated that doses of 500–2000 I.U. PMSG, followed by 500 I.U. hCG 48–96 h later induced ovulation in up to 100% of gilts, independent of age and weight. The ovulation rate was generally related to the dose of PMSG, and the ova released were capable of fertilization. However, the efficiency of superovulation depends not only on the method used, but also on animal factors, such as age, body weight, and breed [7]. A slightly higher number

and percentage of hatched embryos collected from gilts after single synchronization in our study may indicate the applicative value of this synchronization and superovulation schedule. This method enables satisfactory numbers of high quality embryos to be obtained compared to embryos collected from gilts after double synchronization. Although double synchronization did not result in higher numbers or a better quality embryos, perhaps hormonal stimulation combined with a diet enhancing insulin secretion (flushing) [14] could improve the number of good quality embryos for biotechnological studies.

In conclusion, a single synchronization and superovulation schedule is sufficient to obtain high numbers of embryos from prepubertal gilts, however, both synchronization methods resulted in recovering a considerable number of degenerated embryos. Moreover, a higher number and percentage of hatched embryos after *in vitro* culture were found among embryos collected from control, non-stimulated gilts compared to gonadotropin-stimulated gilts.

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