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Effect of different estrus synchronization systems on embryo quality in multiparous sows and prepuberal gilts*)

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Summary

The aim of this study was to assessed the differences in embryos quality between the natural estrus and two systems of estrus synchronization in multiparous sows and prepuberal gilts.

In this experiment, multiparous sows (n = 63) and prepuberal gilts (n = 42) were used. The subgroups of these animals were treated with PMSG (1500 U.I.) + hCG (500 U.I.) or PG-600 synchronization systems. These animals were inseminated twice, 24 and 36 h after hCG injection. The control gilts (n = 20) were from the third subgroup and were inseminated two times at 12 h intervals during their natural estrus cycles.

A statistically significant increased number of corpora lutea (CL) and embryos was observed between natural estrus and both synchronization systems in multiparous sows ($p < 0.001$). There were no differences found in the number of degenerative embryos isolated from both ovaries between PMSG + hCG, PG-600, and natural estrus groups in multiparous sows ($p = 0.484$), ($p = 0.279$), ($p = 0.213$), ($p = 0.138$), respectively. However, an increased number of unfertilized oocytes in multiparous sows after treatment with PMSG + hCG as compared to control animals ($p = 0.041$) was observed. A statistically higher number of embryos after treatment with PMSG + hCG was also observed in the separate groups of multiparous sows and prepuberal gilts as compared to PG-600 treated animals. No differences were found, however, in the number of degenerative embryos between those two separate groups of animals after treatment with PMSG + hCG and PG-600 of both ovaries: ($p = 0.175$), ($p = 0.344$), ($p = 0.122$), and ($p = 0.055$), respectively.

It can be suggested that the differences in the number of embryos isolated from both ovaries after these two treatments systems in prepuberal gilts and multiparous sows may be a result of age-dependent different response to gonadotropins and the reproductive competence of these females.

Keywords: estrus, embryos, gonadotropins

The development of reproduction technology is characterized by the increasing *in vitro* production (IVP) methods' influence to produce a higher number of matured oocytes, and an increased developmental competence of embryos obtained with IVP (1, 9, 17, 20-22). However, the efficiency of those methods and the quality of oocytes and embryos obtained is still reduced compared to procedures applied *in vivo*.

Artificial insemination (AI) and estrus synchronization methods have become basic techniques that may lead to increased numbers of high quality embryos obtained from *in vivo* production (29). Clarification of the efficacy of these procedures may be useful in the future development of successful embryo-transfer machinery in pigs, and in the production of genetically modified pigs used as biomedical models for human disease (3, 7, 13, 19, 23, 24, 29, 30). The efficiency of pig reproduction may be enhanced by introducing more effective methods of estrus synchronization. Several

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lines of experiments indicate that an orally active progestin (Regu-mate) fed in the doses of 12.5 to 15 mg per day synchronizes the estrus cycle in cycling gilts (10-12).

However, one of the most frequently used synchronization systems, PG-600, is a single injection of a combination of pregnant mare serum gonadotropin (PMSG) (400 I.U.) and human chorionic gonadotropin (hCG) (200 I.U.). Britt et al. (5) demonstrated that use of this system leads to the induction of fertile estrus cycling in prepuberal gilts. Other experiments indicate that pretreatment of prepuberal gilts with progesterone prior to intramuscular injection of PMSG enhances follicular development and ovulation rate (23). However, the standard and most popular treatment to gain high quality embryos requires the injection of 1000-1500 I.U. of PMSG followed by 500-700 I.U. of hCG. Karalus et al. (16) describe that a combination of 500 I.U. of hCG and 750-1000 I.U. of PMSG leads to a significant induction of ovulation in 120 day old gilts. Results from other experiments demonstrated that a combination of hCG and PG-600 (hCG + PMSG) or single PG-600 treatment induced superovulation in prepuberal miniature pigs (27). The injection of PG-600 was also associated with an increase in the number of corpora lutea (CL), but increasing the dose of this hormone did not alter the proportions of expressing or ovulating estrus cycles (4).

The development of different synchronization systems is still characterized by reduced efficiency compared to natural estrus cycling on account of a different response to hormonal treatment between multiparous sows and prepuberal gilts. Therefore, the aim of this study was to compare different estrus synchronization systems (PMSG + hCG, and PG-600) and embryo quality isolated from both ovaries obtained from multiparous sows and prepuberal gilts, and to present the differences in ovarian activity in response to exogenous gonadotropin stimulation in age-dependent groups of animals.

Material and methods

This study was performed on two general groups of (cross landrace) female pigs. The first group was composed of sows (n = 63, age: 2.5-5 years, weight: 150-220 kg and having had 3-5 pregnancies with a mean of 11 piglets). The prepuberal gilts (n = 42, age: 140-160 days, weight: 95-100 kg) were in the second group. A total number of 105 animals were used in this study.

Classification of animals. Prepuberal gilts. The group of prepuberal gilts was divided into two subgroups corresponding to different superovulation procedures. The gilts from the first subgroup (n = 21) were synchronized and superovulated by intramuscular injection of 1500 I.U. of PMSG (Folligon, Intervet), and subsequently after 72 h 500 I.U. of hCG (Chorulon, Intervet). These gilts were inseminated twice, at 24 and 36 hours after hCG injection. The gilts from the second subgroup of prepuberal gilts (n = 21) were injected with PG-600, (400 I.U. of PMSG and 200 I.U. of hCG), (Intervet). These animals were inseminated twice, at 24 and 36 h after hCG injection (29).

Multiparous sows. The group of multiparous sows was divided into three subgroups. The first subgroup included sows (n = 21) that were superovulated by using 1500 I.U. of PMSG (Folligon, Intervet) 24 h after weaning. After 72 h, the sows were injected with 500 I.U. of hCG (Chorulon, Intervet). Both hormones were given by intramuscular injection. The sows were inseminated at 24 and 36 h after injection of hCG. The second subgroup of sows (n = 21) was injected with PG-600 (400 I.U. of PMSG and 200 I.U. of hCG) (Intervet) 24 h after weaning. These animals were inseminated twice, at 24 and 36 h after hCG injection. The control sows (n = 21) from the third subgroup were inseminated two times at 12 h intervals during their natural estrus cycles. The multiparous sows used in this study were assigned to the treatments based on their body weight.

The number of ovulated follicles was evaluated from the number of CLs present on the surface of both ovaries. During estrus induction, sows were group-housed in an open-front building with an operational curtain. Following estrus expression, they were moved into an environmentally controlled facility and placed in stalls for insemination. Sows were checked for estrus twice daily by providing them with fence--line contact with a mature boar for a minimum of 15 min. The estrus duration was defined as the first time standing was observed to the last time when the standing response was detected. Animals from all of these groups were slaughtered on the third day after the first insemination. After slaughter, the reproductive tracts were recovered and transported at 37°C to the laboratory. The embryos were collected from both oviducts by flushing with 40 ml Dulbecco–PBS (Sigma, St. Louis, MO, USA) warmed to 38°C. The embryos were counted and investigated morphologically under a stereoscopic microscope. All of the flushed embryos were classified according to the five-grade scale proposed by Veeck (31) with our own modifications. The number of ovulated follicles was also evaluated from the number of CL in both ovaries. The classification of embryos was based on the number of blastomeres, which represented the cleaving status of the embryo. The embryos were graded as follows: grade 5-embryos with blastomeres of equal size and no cytoplasmic fragmentation; grade 4-embryos with blastomeres of equal size and minor cytoplasmic fragmentation; grade 3-embryos with blastomeres of distinctly unequal size and no or little cytoplasmic fragmentation; grade 2-degenerated embryos with blastomeres of equal or unequal size and significant cytoplasmic fragmentation; grade 1-unfertilized oocytes.

Statistical analysis. Results were estimated using one-way analysis of variance (ANOVA) with Newman-Keule's *post-hoc* test. $p \leq 0.05$ was determined as the level of significance. The experiments were approved by the local Ethics Committee.

Results and discussion

This study was based on three experimental designs. The first design demonstrated differences in the number of CL and embryo grades from 5 to 1 after treatment with PMSG + hCG and PG-600 between sows and prepuberal gilts. The second design compared differences in the number of CL and embryo grades based on the same parameters between PMSG + hCG, PG-600, and natural estrus cycles. The third design was based on a comparison between PMSG + hCG and PG-600 synchronization systems in the separate groups of multiparous sows and prepuberal gilts.

Tab. 1. Association between embryo quality and different ovulation synchronization systems in multiparous sows and prepuberal gilts

Embryo quality		Multiparous sows			Prepuberal gilts		Significance (*P*)			
		PMSG + hCG Total (mean ± SEM)	PG-600 Total (mean ± SEM)	Natural estrus Total (mean ± SEM)	PMSG + hCG Total (mean ± SEM)	PG-600 Total (mean ± SEM)	a	b	c	d
CL	LO	113 (5.38 ± 0.39)	76 (3.80 ± 0.40)	146 (6.95 ± 0.51)	289 (14.2 ± 1.19)	212 (10.1 ± 0.50)	< 0.001	< 0.001	0.01	< 0.001
	RO	116 (5.52 ± 0.52)	53 (2.65 ± 0.31)	106 (5.04 ± 0.49)	249 (11.8 ± 0.72)	201 (9.57 ± 0.57)	< 0.001	< 0.001	0.256	< 0.001
5	LO	17 (0.81 ± 0.17)	7 (0.37 ± 0.11)	39 (1.85 ± 0.39)	100 (4.76 ± 0.73)	57 (2.71 ± 0.39)	< 0.001	< 0.001	0.01	< 0.001
	RO	9 (0.82 ± 0.42)	4 (0.21 ± 0.12)	8 (0.38 ± 0.14)	60 (2.85 ± 0.51)	42 (2.10 ± 0.44)	< 0.001	0.0003	0.409	0.188
4	LO	9 (0.42 ± 0.14)	15 (0.75 ± 0.210)	33 (1.57 ± 0.22)	58 (2.76 ± 0.38)	46 (2.19 ± 0.31)	< 0.001	0.0003	< 0.001	0.006
	RO	20 (0.95 ± 0.20)	3 (0.15 ± 0.081)	28 (1.33 ± 0.23)	50 (2.38 ±0.32)	40 (1.90 ± 0.40)	0.0004	0.0002	0.111	< 0.001
3	LO	22 (1.04 ± 0.22)	19 (1.00 ± 0.26)	33 (1.57 ± 0.25)	40 (1.90 ± 0.31)	45 (2.14 ± 0.26)	0.016	0.0027	0.064	0.068
	RO	17 (0.81 ± 0.20)	6 (0.33 ± 0.12)	25 (1.19 ± 0.20)	39 (1.85 ± 0.21)	79 (1.52 ± 0.32)	0.0007	0.0013	0.095	< 0.001
2	LO	28 (1.33 ± 0.28)	20 (1.00 ± 0.20)	25 (1.31 ± 0.31)	61 (2.90 ± 0.30)	47 (2.23 ± 0.47)	0.0003	0.0123	0.484	0.213
	RO	28 (1.33 ± 0.25)	24 (1.20 ± 0.20)	32 (1.52 ± 0.20)	55 (2.61 ± 0.32)	36 (1.71 ± 0.44)	0.001	0.1543	0.279	0.138
1	LO	8 (0.38 ± 0.140)	1 (0.05 ± 0.048)	3 (0.14 ± 0.100)	2 (0.09 ± 0.06)	0	0.042	0	0.096	0.214
	RO	5 (0.25 ± 0.096)	2 (0.10 ± 0.090)	1 (0.05 ± 0.048)	2 (0.09 ± 0.06)	0	0.101	0	0.041	[b]0.329

Explanations: a – statistical difference of PMSG + hCG synchronization systems between multiparous sows and prepuberal gilts; b – statistical difference of PG-600 synchronization systems between multiparous sows and prepuberal gilts; c – statistical difference between natural estrus cycling and PMSG + hCG synchronization system in multiparous sows; d – statistical difference between natural estrus cycling and PG-600 synchronization system in multiparous sows; LO – left ovary; RO – right ovary. Numbers are given as total (mean ± SEM). CL – number of corpora lutea. Numbers from 5 to 1 represent the graded scale of embryo quality proposed by Veeck et al. (31), with our own modifications

Experimental design 1. Association between PMSG + hCG synchronization system and embryo quality in multiparous sows and prebuberal gilts. The authors found a statistically decreased number of CL on left and right ovaries isolated from multiparous sows as compared to prepuberal gilts, with 113 (5.38 ± 0.39) to 289 (14.2 ± 1.19) in the left and 116 (5.52 ± 0.52) to 249 (11.8 ± 0.72) in the right ($p \leq 0.001$), respectively. We also found statistically significant differences in the quality of embryos, graded as 5 and 4, between multiparous sows and prepuberal gilts, from both ovaries, with 17 (0.81 ± 0.17) to 100 (4.76 ± 0.73) on the left and 9 (0.82 ± 0.42) to 60 (2.85 ± 0.51) on the right for grade 5, and 9 (0.42 ± 0.14) to 58 (2.76 ± 0.38) on the left and 20 (0.95 ± 0.20) to 50 (2.38 ± 0.32) on the right for grade 4 ($p \leq 0.001$), respectively (tab. 1). We described a significantly decreased number of embryos graded as 3 isolated from both ovaries of multiparous sows compared to prepuberal gilts, namely 22 (1.04 ± 0.22) to 40 (1.90 ± 0.31) on the left and 17 (0.81 ± 0.20) to 39 (1.85 ± 0.21) on the right ($p = 0.016$), ($p = 0.0007$), respectively. The number of degenerative embryos (graded as 2) was significantly higher in prepuberal gilts as compared to multiparous sows, at 61 (2.90 ± 0.30) to 28 (1.33 ± 0.28) on the left and 55 (2.61 ± 0.32) to 28 (1.33 ± 0.25) on the right ($p = 0.0003$), ($p = 0.001$), respectively. We did not observe statistically significant differences in the number of unfertilized oocytes between multiparous sows and prepuberal gilts on the right ovary; namely 5 (0.25 ± 0.096) to 2 (0.09 ± 0.06), ($p = 0.101$), (tab. 1). However, we found a higher number of unfertilized embryos in sows on the left ovary, specifically 8 (0.38 ± 0.14) to 2 (0.09 ± 0.06), ($p = 0.042$).

Association between PG-600 synchronization system and embryo quality in multiparous sows and prepuberal gilts. The number of CL isolated from both ovaries was significantly increased in prepuberal gilts as compared to multiparous sows, with 212 (10.1 ± 0.50) to 76 (3.8 ± 0.40) in the left ovary and 201 (9.57 ± 0.57) to 53 (2.65 ± 0.31) in the right ovary ($p = 0.001$), respectively. We found a higher number of embryos graded as 5 and 4 in prepuberal gilts compared to multiparous sows, namely 57 (2.71 ± 0.39) to 7 (0.37 ± 0.11) on the left and 42 (2.1 ± 0.44) to 4 (0.21 ± 0.12) on the right, and 46 (2.19 ± 0.31) to 15 (0.75 ± 0.21) on the left and 40 (1.90 ± 0.40) to 3 (0.15 ± 0.081) on the right ($p \leq 0.001$), ($p \leq 0.001$), respectively (tab. 1). We also observed statistical differences in the quality of embryos graded as 3 and degenerative embryos between multiparous sows and prepuberal gilts, such that there were 19 (1 ± 0.26) to 45 (2.14 ± 0.26) on the left and 6 (0.33 ± 0.12) to 79 (1.52 ± 0.32) on the right, compared to 20 (1 ± 0.20) to 47 (2.23 ± 0.47) on the left ($p = 0.0027$), ($p = 0.0013$), ($p = 0.0123$), respectively. However we did not find any differences in the number of degenerative embryos in the right ovary, 24 (1.2 ± 0.20) to 36 (1.71 ± 0.44), ($p = 0.1543$), (tab. 1). We did not detect unfertilized oocytes in prepuberal gilts after treatment with PG-600.

Experimental design 2. Association between natural estrus cycle and PMSG + hCG synchronization system in multiparous sows. The authors have observed statistically significant differences in the number of CL and quality of embryos graded as 5 between PMSG + hCG and natural estrus cycling on the left ovary only of the multiparous sows, 113 (5.38 ± 0.39) to 146 (6.95 ± 0.51), 17 (0.81 ± 0.17) to 39 (1.85 ± 0.39), ($p = 0.01$), ($p = 0.01$), respectively (tab. 1). We did not observe differences on the right ovary, 116 (5.52 ± 0.52) to 106 (5.04 ± 0.49), 9 (0.82 ± 0.42) to 8 (0.38 ± 0.14), ($p = 0.256$), ($p = 0.409$), respectively.

The authors have found an increased number of embryos graded as 4 only on the left ovary of the control group, as compared to PMSG + hCG system in the multiparous sows, 33 (1.57 ± 0.22) to 9 (0.42 ± 0.14), ($p \leq 0.001$). However, we did not observe those differences on the right ovary of these groups, 20 (0.95 ± 0.20) to 28 (1.33 ± 0.23), ($p = 0.111$). The authors did not find statistical differences in the number of embryos graded as 3, or the number of degenerative embryos between PMSG + hCG system and natural estrus cycling, on both ovaries, with 22 (1.04 ± 0.22) to 33 (1.57 ± 0.25) on the left and 17 (0.81 ± 0.20) to 25 (1.19 ± 0.20) on the right, compared to 28 (1.33 ± 0.28) to 25 (1.31 ± 0.31) on the left and 28 (1.33 ± 0.25) to 32 (1.52 ± 0.20) on the right, ($p = 0.064$), ($p = 0.095$), ($p = 0.484$), ($p = 0.279$), respectively (tab. 1). We also did not see differences in the number of unfertilized oocytes on the left ovary, 8 (0.38 ± 0.14) to 3 (0.14 ± 0.10), ($p = 0.096$). However, we observed a statistically increased number of unfertilized oocytes on the right ovary, 5 (0.25 ± 0.096) to 1 (0.05 ± 0.048), ($p = 0.041$), (tab. 1).

Association between natural estrus cycling and PG-600 synchronization system in multiparous sows. The authors have found a significantly increased number of CL in both ovaries isolated from multiparous sows after natural estrus cycling as compared to PG-600 system, with 146 (6.95 ± 0.51) to 76 (3.8 ± 0.40) on the left and 106 (5.04 ± 0.49) to 53 (2.65 ± 0.31) on the right ($p \leq 0.001$), ($p \leq 0.001$), respectively.

An increased number of embryos graded as 5 after natural estrus cycling as compared to PG-600-treated multiparous sows were also observed, but only on the left ovary, 39 (1.85 ± 0.39) to 7 (0.37 ± 0.11), ($p \leq 0.001$) respectively. However, we did not find any differences on the right ovary, 8 (0.38 ± 0.14) to 4 (0.21 ± 0.12), ($p = 0.188$), respectively (tab. 1). The number of embryos graded as 4 was significantly increased on both ovaries in the control group compared to the multiparous sows treated with PG-600, namely 33 (1.57 ± 0.22) to 15 (0.75 ± 0.21) on the left and 28 (1.33 ± 0.23) to 3 (0.15 ± 0.081) on the right ($p = 0.006$, $p \leq 0.001$, respectively). The number of embryos graded as 3 isolated from the left ovary was not statistically different between natural estrus and PG-600-treated multiparous sows, 33 (1.57 ± 0.25) to 19 (1 ± 0.26), ($p = 0.068$), respectively (tab. 1), although also the authors found a statistical difference on the right ovary, 25 (1.19 ± 0.20) to 6 (0.33 ± 0.12), ($p \leq 0.001$), respectively. We did not observe any differences in the number of degenerative embryos and unfertilized oocytes between PG-600 system and natural estrus cycling, with 20 (1 ± 0.20) to 25 (1.31 ± 0.31) on the left and 24 (1.2 ± 0.20) to 32 (1.52 ± 0.20) on the right, compared to 1 (0.05 ± 0.048) to 3 (0.14 ± 0.10) on the left and 2 (0.1 ± 0.09) to 1 (0.05 ± 0.048) on the right ($p = 0.213$), ($p = 0.138$), ($p = 0.214$), ($p = 0.329$), respectively (tab. 1).

Experimental design 3. Comparison of embryo quality between PMSG + hCG and PG-600 synchronization systems in multiparous sows. The authors found statistical differences in the number of corpora lutea in multiparous sows between these two synchronization systems on both ovaries, 113 (5.38 ± 0.39) to 76 (3.8 ± 0.40) on the left and 116 (5.52 ± 0.52) to 53 (2.65 ± 0.31) on the right ($p = 0.0043$), ($p \leq 0.001$), respectively. We did not find any differences in the number of embryos graded as 5 on the right ovary, 9 (0.82 ± 0.42) to 4 (0.21 ± 0.12), ($p = 0.131$), (tab. 2). However, we observed differences on the left ovary, 17 (0.81 ± 0.17) to 7 (0.37 ± 0.11), ($p = 0.022$). The number of embryos graded as 4 were statistically different between these two synchronization systems only on the right ovary, 20 (0.95 ± 0.20) to 3 (0.15 ± 0.081), ($p \leq 0.001$). However, we did not observe any difference in the left ovary, 9 (0.42 ± 0.14) to 15 (0.75 ± 0.21), ($p = 0.114$), respectively. We did not see differences in the number of embryos graded as 3 on the left ovary, 22 (1.04 ± 0.22) to 19 (1 ± 0.26), ($p = 0.447$), although we observed differences on the right ovary, 17 (0.81 ± 0.20) to 6 (0.33 ± 0.12), ($p = 0.030$), respectively.

The authors also did not find statistical differences in the number of degenerative embryos on both ovaries, with 28 (1.33 ± 0.28) to 20 (1 ± 0.20) on the left and 28 (1.33 ± 0.25) to 24 (1.2 ± 0.20) on the right, ($p = 0.175$), ($p = 0.344$), respectively (tab. 2). We did not observe differences in the number of unfertilized oocytes on the right ovary, 5 (0.25 ± 0.096) to 2 (0.1 ± 0.09), ($p = 0.147$),

Tab. 2. Association between PMSG + hCG and PG-600 synchronization systems in multiparous sows and prepuberal gilts

Embryo quality		Multiparous sows		Prepuberal gilts		Significance (*P*)	
		PMSG + hCG Total (mean ± SEM)	PG-600 Total (mean ± SEM)	PMSG+hCG Total (mean±SEM)	PG-600 Total (mean±SEM)	a	b
CL	LO	113 (5.38 ± 0.39)	76 (3.80 ± 0.40)	289 (14.2 ± 1.19)	212 (10.1 ± 0.50)	0.0043	0.0019
	RO	116 (5.52 ± 0.52)	53 (2.65 ± 0.31)	249 (11.8 ± 0.72)	201 (9.57 ± 0.57)	< 0.001	0.009
5	LO	17 (0.81 ± 0.17)	7 (0.37 ± 0.11)	100 (4.76 ± 0.73)	57 (2.71 ± 0.39)	0.022	0.01
	RO	9 (0.82 ± 0.42)	4 (0.21 ± 0.12)	60 (2.85 ± 0.51)	42 (2.10 ± 0.44)	0.131	0.140
4	LO	9 (0.42 ± 0.14)	15 (0.75 ± 0.210)	58 (2.76 ± 0.38)	46 (2.19 ± 0.31)	0.114	0.130
	RO	20 (0.95 ± 0.20)	3 (0.15 ± 0.081)	50 (2.38 ± 0.32)	40 (1.90 ± 0.40)	< 0.001	0.184
3	LO	22 (1.04 ± 0.22)	19 (1.00 ± 0.26)	40 (1.90 ± 0.31)	45 (2.14 ± 0.26)	0.447	0.284
	RO	17 (0.81 ± 0.20)	6 (0.33 ± 0.12)	39 (1.85 ± 0.21)	79 (1.52 ± 0.32)	0.030	0.195
2	LO	28 (1.33 ± 0.28)	20 (1.00 ± 0.20)	61 (2.90 ± 0.30)	47 (2.23 ± 0.47)	0.175	0.122
	RO	28 (1.33 ± 0.25)	24 (1.20 ± 0.20)	55 (2.61 ± 0.32)	36 (1.71 ± 0.44)	0.344	0.055
1	LO	8 (0.38 ± 0.140)	1 (0.05 ± 0.048)	2 (0.09 ± 0.06)	0	0.021	0
	RO	5 (0.25 ± 0.096)	2 (0.10 ± 0.090)	2 (0.09 ± 0.06)	0	0.147	0

Explanations: a – statistical differences between PMSG + hCG and PG-600 synchronization systems in multiparous sows; b – statistical differences between PMSG + hCG and PG-600 synchronization systems in prepuberal gilts; LO – left ovary; RO – right ovary

but we found differences on the left ovary, 8 (0.38 ± 0.14) to 1 (0.05 ± 0.048), (p = 0.021) respectively.

Comparison of embryo quality between PMSG + hCG and PG-600 synchronization systems in prepuberal gilts. The authors have found statistical differences in the number of CL on both ovaries, namely 289 (14.2 ± 1.19) to 212 (10.1 ± 0.50) on the left and 249 (11.8 ± 0.72) to 201 on the right (9.57 ± 0.57), (p = 0.0019), (p = 0.009), respectively (tab. 2). We observed an increased number of embryos graded as 5 only on the left ovary, 100 (4.76 ± 0.73) to 57 (2.71 ± 0.39), (p = 0.01). On the right ovary, 60 (2.85 ± 0.51) to 42 (2.1 ± 0.44), (p = 0.140) did not show a statistical difference. We did not see differences in the number of embryos graded as 4, 3 and degenerative embryos on both ovaries, namely 58 (2.76 ± 0.38) to 46 (2.19 ± 0.31) on the left and 50 (2.38 ± 0.32) to 40 (1.90 ± 0.40) on the right, 40 (1.90 ± 0.31) to 45 (2.14 ± 0.26) on the left and 39 (1.85 ± 0.21) to 32 (1.52 ± 0.32) on the right, 61 (2.90 ± 0.30) to 47 (2.23 ± 0.47) on the left and 55 (2.61 ± 0.32) to 36 (1.71 ± 0.44) on the right, (p = 0.130), (p = 0.184), (p = 0.284), (p = 0.195), (p = 0.122), (p = 0.055), respectively (tab. 2). After treatment of prepuberal gilts with PG-600, we did not find unfertilized oocytes.

Since Casida et al. (8) first demonstrated the induction of ovulation after injection of exogenous gonadotropins, hormonal treatments have undergone several modifications. Britt et al. (5) proved that a single injection of PG-600 leads to the induction of estrus cycling in prepuberal gilts, and the number of pigs born alive or dead were similar for the PG-600 and control animals. Knox et al. (18) compared the effect different doses of PG-600 have on estrus and ovulatory responses of prepuberal gilts. They proved that administration of PG-600 leads to induction of estrus cycling in prepuberal gilts as compared to controls. Moreover, they demonstrated that the number of CL was not influenced when the site of injection was either the flank or the neck during treatment with PG-600.

The authors investigated the number of CL on both ovaries after administration of two different synchronization systems, PSMG + hCG and PG-600, and found statistically increased numbers of CL on both ovaries in multiparous sows and prepuberal gilts after treatment with PMSG + hCG when compared to PG-600. Thus, we proved that PMSG + hCG gives the best results in the number of CL compared to PG-600, which may be explained by higher doses of gonadotropins that induced estrus cycling and ovulation in both groups of animals (2, 11, 14, 25, 26). Holtz and Schlieper (14) described that prepuberal domestic gilts treated with 1, 2, or 3 vials of PG-600 lead to an increased number of residual follicles in a dose dependent manner. By contrast, results obtained by Shimatsu et al. (27) do not demonstrate a relationship between dosage of PG-600 and the number of ovulations. However, they show that combining PG-600 with 100 I.U. of hCG resulted in a sufficient number of ovulations.

The authors proposed that each of the two studied synchronization systems has a different effect on the number of embryos in multiparous sows and prepuberal gilts, although there were some individual variations. In the present study, it was demonstrated that the greater differences in the number of CL and the best qualities of embryos, graded as 5 and 4, were found usually on the right ovary in multiparous sows after treatment with PMSG + hCG (tab. 2). This may be explained by an increased activity of the right ovary in response to the inducible effect of an increased dosage of gonadotropins. Contrary to the results obtained by Ziecik et al. (34), we did not observe any differences in the number of degenerative embryos between PMSG + hCG and PG-600 synchronization systems and natural estrus cycling in multiparous sows and prepuberal gilts, which suggests that these systems and natural estrus cycling have a similar effect on degenerative processes in embryos, and that this effect is not hormone dose dependent (tab. 2). Moreover, the authors have suggested that neither of the synchronization systems based on exogenous gonadotropins affects oocyte growth and embryo development. It was also proved by Kaneko et al. (15) that gonadotropins accelerate follicular growth and increase the number of recovered full-sized oocytes with meiotic and maturation competence.

On the other hand, an increased number of unfertilized oocytes *in vivo* after PMSG + hCG in multiparous sows as compared to natural estrus cycling may be explained by the individual features and decreased fertilization potential of those animals, which results from *in vivo* treatment with an increased dose of gonadotropins, and by the increasing age of these females, which did not affect oocyte growth but may result in several disturbances in gamete interaction and during fertilization (6, 33), (tab. 1). Similarly, Baker et al. (2) demonstrated that induction of superovulation by using high doses of equine chorionic gonadotropin (eCG) and hCG in prepuberal domestic gilts was associated with an increased number of unfertilized oocytes and polyspermic embryos. Contrary to these results, Kaneko et al. (15) showed that the addition of gonadotropins to *in vitro* matured oocytes increased the number of successfully fertilized gametes as compared to controls. Their study proved that *in vitro* conditions give different results in comparison to experiments of gonadotropin treatment *in vivo*.

Moreover, the authors found an increased number of CL and all qualities of embryos graded from 5 to 3 in multiparous sows after natural estrus cycling as compared to both synchronization systems. These results proved that neither PMSG + hCG nor PG-600 give similar results to natural estrus cycling. Thus, exogenous stimulation with gonadotropins in pigs is still unsatisfactory and further research is necessary to determine methods that will allow treated animals to reach almost the same level of fertility as natural estrus cycling animals. However, no differences in the number of degenerative embryos were observed. This indicates that differences between synchronization systems and natural estrus cycling might result from different responses to exogenous gonadotropins *in vivo* that do not affect embryo growth (18). These results were proved, on a sheep model, by Veiga-

-Lopez et al. (32), who found that the presence of CL at the start of superovulatory treatment may have a protective effect on embryonic viability and decrease the degeneration of embryos. Our results clearly show that an increased dose of exogenous gonadotropins in the form of PMSG + hCG leads to an increased number of CL, which may inhibit degeneration mechanisms in both multiparous sows and prepuberal gilts (tab. 1).

The authors results demonstrate significant differences in two synchronization systems, PMSG + hCG and PG-600, between multiparous sows and prepuberal gilts, also when comparing the left and right ovary. We also demonstrated differences in the number of CL in both ovaries after the injection of these hormones and the quality of embryos after treatment. The numbers of CL and embryos after treatment with PMSG + hCG in multiparous sows and prepuberal gilts were increased as compared to animals treated with the PG-600 system (tab. 2). The differences in the number of embryos isolated from those two groups after treatment may be due to the reproductive ability of the animals and other animal factors influencing the fertilization ability of oocytes and developmental competence of embryos. This also confirms the hypothesis that individual response to exogenous gonadotropins is in a dose dependent manner (4). These results were also demonstrated by Wiesak et al. (33), who analyzed the effect of PMSG + hCG treatment and natural estrus cycling on follicular development between two varying-age groups of females. They showed that the ovaries of animals injected with gonadotropins are functionally different from the ovaries of mature females, and may result in different reproductive competence of these animals. An increased number of CL and embryos isolated from prepuberal gilts as compared to multiparous sows may be the result of different individual responses to PMSG + hCG and the age of the animals. The results of this study require further research with larger groups of females, and with the use of recombinant human (rh) FSH and LH *in vivo*, which give the best results in *in vitro* pig embryo production when substituting them for PMSG and hCG (28).

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