Animal Science Papers and Reports vol. 32 (2014) no. 1, 5-13
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Effect of hCG and intravaginal application of estradiol and prostaglandin E_2 on pregnancy rate and litter size in gilts and sows*

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(Received August 14, 2013; accepted November 19, 2013)

Three separate experiments were performed on fifty pregnant gilts (Experiment 1 and 2) and forty primiparous sows (Experiment 3) to examine the effect of human *chorionic gonadotropin* (hCG) administration on day 12 (Experiments 1 and 2) or 20 (Experiments 2 and 3) of pregnancy and intravaginal application of estradiol alone or estradiol + prostaglandin E_2 (PGE_2) on days 17-23 (Experiment 3) of pregnancy on pregnancy rate and litter size and weight at birth and weaning. None of the treatments affected the pregnancy rate which ranged between 90 and 80% in control and treated groups. In Experiment 1 no differences in the number of piglets born (13.2±1.1 or 13.0±0.8) and weaned between control and hCG group were noticed.

In Experiment 2 hCG treatment increased the total number of piglets born. For treatment on day 12 this number amounted to 15.1±1.3, for day 20 – 16.4±1.4, while for control gilts 13.0±1.5. The similar trend occurred in the number of piglets born alive. The remaining indicators of piglets survival did not vary between groups.

Though there were no significant differences in the number of total piglets born between groups of sows in Experiment 3, a trend to the increased number of piglets weaned was found in groups with intravaginal treatment of estradiol and PGE_2 (P= 0.10) or hCG administered on day 20 of

*Financed by the National Centre for Research and Development in Poland (NCRD), grant No. 12- 0039-10
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pregnancy. The study revealed, for the first time, that hCG or intravaginal estradiol and PGE_2 administration during early pregnancy did not affect the pregnancy rate in gilts and sows and can be beneficial for litter size.

KEY WORDS: estradiol / hCG / litter size / PGE_2 / pig / pregnancy

The establishment of pregnancy in pigs depends on the continuous maintenance of corpora lutea and sufficient supply of progesterone, which concentration is positively correlated with embryonic survival during the first month of gestation [Jindal *et al.* 1997]. A minimum of 4 ng/ml of progesterone in plasma has been found to be essential for maintenance of pregnancy. However, supplementation with progesterone or estradiol during the early pregnancy yielded an equivocal results [Mao and Foxcroft 1998, Day *et al.* 1969, Morrissette *et al.* 1963, Schultz *et al*. 1966, Wildt *et al.* 1976]. In addition, the premature time exposure (days 9-10 post-insemination) of the uterus to estradiol (E_2) altered the expression of some endometrial genes and led to the pregnancy failure [Geisert *et al*. 2006].

The intravaginal application of E_2 on days 2-6 and then of progesterone on days 12-17 of pregnancy increased weight and size of the embryos in gilts on day 30 of pregnancy [Chlopek *et al*. 2008]. The similar intravaginal treatment with combination of E_2 and PGE_2 on days 11-16 of the estrous cycle could prolong corpora lutea function by 40% in the treated gilts [Przygrodzka *et al*. 2014].

On the other hand, the beneficial effect on embryo survival after hCG administration was observed in pigs [Tilton *et al*. 1989]. Similar study performed in our laboratory confirmed that a single administration of hCG on day 12 of pregnancy affected conceptus development through enhanced angiogenesis in corpora lutea and endometrium and decreased luteal apoptosis in early pregnant pigs [Bolzan *et al*. 2013].

In the available literature there is a lack of information about the effect of hCG treatment as well as intravaginal application of E_2 and PGE_2 during the early pregnancy on the course of the whole pregnancy and litter fate until weaning of pigs.

Majority of the studies on the effect of various hormones on embryo survival in pigs performed so far, concerned exclusively the period up to day 30 of pregnancy [Webel *et al*. 1975, Tilton *et al*. 1989, Pope *et al*. 1986, Chlopek *et al.* 2008, Bolzan *et al*. 2013] and even if some beneficial data were obtained, it was still uncertain whether this effect was maintained until the end of pregnancy. Therefore, the aim of this study was to evaluate the effect of hCG administration and intravaginal applications of E_2 or/and PGE_2 during the early pregnancy on the litter size and piglets survival until weaning.

Material and methods

Experiments were conducted on fifty pregnant gilts and forty pregnant sows, on three pig farms in Poland. Gilts were inseminated in second or third estrus. On day 12 (Experiment 1 and 2) or day 20 (Experiment 2) of pregnancy females were challenged

with hCG (Chorulon, INTERVET, Schering-Plough Animal Health) or saline (control groups). The primiparous sows were inseminated during the post-weaning estrus, administered with hCG and intravaginally treated with combination of E_2 and/or PGE_2 (Experiment 3).

Experiment 1

Crossbred gilts of PenArLan-Naima (commercial Farm A) were randomly divided into two groups as follows: hCG group (n=10) intramuscularly given 500 IU of hCG and control group (n=10) injected with 2 ml of saline on day 12 of pregnancy.

Experiment 2

Thirty crossbred gilts (PIC x Danbred) on commercial Farm B were divided into three groups (n=10 per group): hCG-12 (500 IU of hCG injected on day 12 of pregnancy), hCG-20 (500 IU of hCG injected on day 20 of pregnancy) and control (injection of 2 ml of saline) on day 20. In both experiments gilts were ultrasonographically examined for pregnancy on day 30 and 60 of pregnancy and the number of alive and stillbirth piglets was determined at birth. The number of weaned piglets on days 28-29 of lactation and the mean litter weight at birth and weaning were determined.

Experiment 3

The crossbred primiparous sows were inseminated during the first post-weaning estrus and divided into four groups (n=10 per group). Sows from Groups I and II were daily intravaginally treated with 0.4 mg of estradiol-17β (E_2) (I) and 0.4 mg of E_2 + 2 mg of prostaglandin E_2 (PGE_2) (B) on days 17-23 of pregnancy. Vaginal suppositories containing E_2 (SIGMA-ALDRICH, USA) were prepared using cocoa butter (*cacao oleum*; PHARMA COSMETICS, Poland) according to the method described by Price *et al*. [1983] and stored at +4°C. Additionally, suppositories with PGE_2 (CAYMAN CHEMICALS, Ann Arbor, MI, USA) were made using WITEPSOL H-15 base. First, adequate amount of WITEPSOL H-15 was mixed with PGE_2 using UNGUATOR® e/s and then casted to the forms. Afterwards, the suppositories were chilled to avoid sedimentation of solids and further stored at -20°C. Before application, suppositories with PGE_2 were kept at room temperature for 15 min.

Sows from Group III were challenged with single intramuscular injection of hCG (750 I.U) on day 20 of pregnancy. Animals from Group IV (Control) were intravaginally treated with placebo (*cacao oleum*; PHARMA COSMETICS) and single injection of saline (2 ml) on day 20 of pregnancy. Ultrasound examination for the embryos presence was performed on days 22-24 and 30-35 of pregnancy. The number of born (alive and stillbirth), the number of weaned piglets on day 29 of lactation and their mean weight at birth and weaning were recorded.

Statistical

The data were expressed as means ± SEM of values obtained in three experiments. One way ANOVA followed by Bonferroni's *post hoc* test (GraphPad Prism v. 5.0, GraphPad Software, San Diego, CA, USA) was applied for values obtained in Experiment II and III. The data from Experiment I were compared by t test GraphPad Prism v. 5.0, GraphPad Software, San Diego, CA, USA).

Results and discussion

Experiment 1

The pregnancy rate in both groups of gilts (measured as the percent of pregnancies with regard to the performed insemination) was 80%. The USG investigations on days 22-24 after artificial insemination (AI) showed presence of fetuses in 9 gilts of both hCG and control group. However, the second USG study done 10 days later did not confirm pregnancy in one of hCG given (No 940) and one control gilt (No 952). The total number of piglets born (alive and dead) did not differ between control (13.25±1.13) and hCG group (13.00±0.80). No differences were found in the number of alive and dead piglets calculated separately (Tab. 1). The control of litters after weaning did not show any significant differences in their size and weight (Tab. 1).

Table 1. Effects of treatment on litter size and piglet weight in Experiment 1 (means±SEM)

Piglets	Group	
	control (n=8)	hCG (n=8)
Total born (n)	13.25±1.13	13.00±0.80
Born alive (n)	10.75±1.16	10.13±0.61
Stillbirth (n)	1.13±0.44	2.00±0.91
Piglet weight at birth (kg)	1.20±0.03	1.27±0.03
Weaned (n)	9.63±0.73	9.75±0.45
Piglet weight at weaning (kg, lactation 29 days)	7.34±0.18	7.62±0.19

Experiment 2

The pregnancy rates on the farm were as follows: 90 (control group), 80 (hCG-12) and 80% (hCG-20). In one gilt from Group hCG-12 and two gilts from Group hCG-20 estrus behavior was noticed 21 days after insemination.

Results of Experiment 2 are presented in Table 2 and Figure 1. There is a tendency to the increased number of piglets in litters of gilts challenged with hCG on day 12 and 20 of the pregnancy. Significant differences occurred in the total number of piglets born in group hCG – 20 *vs.* control (16.4±1.4 *vs.* 13.01±1.5, respectively; $P<0.05$). The remaining indicators of piglets survival (litter size and weight) did not vary between groups.

Table 2. Effects of treatment on litter size and piglet weight in Experiment 2 (means±SEM)

Piglets	Group		
	control (n=9)	hCG – 12 (n=8)	hCG – 20 (n=8)
Total born (n)	13.0±1.5	15.1±1.3	16.4±1.4*
Born alive (n)	11.6±1.2	13.4±1.2	14.60±0.7
Stillbirth (n)	0.9±0.4	0.6±0.3	1.0±0.6
Piglet weight at birth (kg)	1.3±0.1	1.3±0.1	1.3±0.1
Weaned (n)	11.6±0.4	12.3±0.4	11.9±0.4
Piglet weight at weaning (kg, lactation 28 days)	7.7±0.2	6.9±0.2	6.7±0.3

* P<0.05 *vs.* control.

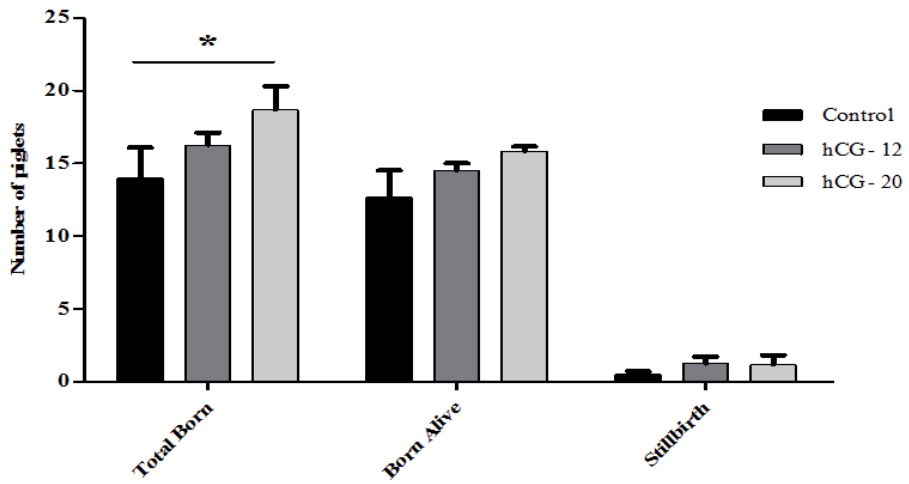

Fig. 1. Effect of hCG administration on day 12 (hCG-12) and 20 (hCG-20) of pregnancy in gilts on number of total born alive and dead born of piglets. Control gilts received 2 ml of saline (mean ±SEM; *P<0.05).

Experiment 3

Pregnancy rate of primiparous sows was 90 (Group I and IV) and 80% (Group II and III), respectively. Interestingly, pregnancy was identified in all sows from Group II and III, but not confirmed in two females from both groups on day 35 after AI. The total number of piglets born ranged from 15.1±0.7 (Group III) to 14.7±1.7 (Group IV) and the number of alive from 12.8±0.5 (Group III) to 11.1±1.8 (Group D, Tab. 3). The highest number of stillbirths was found in sows from Group IV (2.4±1.3) and lowest in Group I (1.0±0.3), but intergroup difference was not significant. Also the lowest number of weaned piglets was noticed in Group IV (placebo) and a tendency to the increased number of weaned piglets was found in groups with intervaginal treatment with estradiol and PGE_2 or hCG on day 20 of pregnancy (P=0.10).

Table 3. Effect of intravaginal administration of estradiol 17β (Group I), estradiol 17β and PGE2 (Group II) on days 17-23, hCG administration on day 20 (Group III) of pregnancy respectively; intravaginal administration of placebo and saline treatment (Group IV) on litter size at birth and weaning in Experiment 3 (means ±SEM)

Piglets	Group I (n=9)	Group II (n=8)	Group III (n=8)	Group IV (n=9)
Total born (n)	14.57±1.49	14.43±1.04	15.14±0.74	14.71±1.67
Born alive (n)	12.00±0.85	12.43±0.48	12.86±0.46	11.14±1.77
Stillbirth (n)	1.00±0.31	1.71±0.68	1.29±0.47	2.43±1.31
Piglet weight at birth (kg)	1.26±0,04	1.35±0.09	1.22±0.02	1.32±0.03
Weaned (n)	10.86±0.55	11.43±0.43	11.43±0.68	9.43±1.11
Piglet weight at weaning (kg, lactation 29 days)	8.57±0.40	8.03±0.31	7.83±0.17	7.76±0.19

In all experiments performed on three farms both hCG and estradiol/PGE_2 did not have negative effect on the pregnancy rate which varied in control and experimental groups between 90 and 80%.

The results of Experiment 1 did not show the effect of hCG given on day 12 of pregnancy on litter size at birth and at weaning, piglets weight at birth and weaning, and stillbirths. However, the similar Experiment 2, performed on Farm B, demonstrated the significant increase in the number of total piglets born after hCG administration on day 20 of the pregnancy and significant (P = 0.10) increase of the litter size in group of gilts treated with hCG on day 12.

Similarly, the beneficial effect of hCG seems to occur regarding the number of piglets born alive in both groups challenged with hCG. Although the experiments were conducted on gilts of different crossbred lines, control gilts on both farms delivered similar litters concerning the size. However, PIC x Danbred gilts on farm B had higher number of weaned piglets. It is difficult to settle whether better response to hCG challenge on Farm B is farm-specific or depends on genetic background of the animals.

The last experiment was performed on primiparous sows and except hCG administration on day 20 of pregnancy, two experimental groups of sows were challenged with intravaginal administration of estradiol alone and estradiol + PGE_2. Besides that both substances are luteotrophic in pigs, the period of administration was adjusted to the natural increase of embryonic estrogens in sow's blood during the early pregnancy [Geisert *et al.* 1990]. The intravaginal estradiol and PGE_2 treatment applied in cyclic gilts during the mid-luteal phase was able to prolong CL function in 40% of females [Przygrodzka *et al*. 2014]. The applied dose of estradiol was comparable to that which was used by Chlopek *et al*. [2008] and regardless of different ways of administration, doses of estradiol was 10 times lower than applied to trigger pseudopregnancy [Ziecik *et al.* 1986]. Though there were no significant differences identified in the number of total piglets born between groups, a clear tendency to the increased number of born alive and weaned piglets was noticed, especially after

intravaginal treatment with estradiol + PGE_2 and/or hCG administration on day 20 of pregnancy.

Generally, results of our experiments suggest that all the substances presented here and used for gilts and sows (hCG or PGE_2 and estradiol) did not negatively affect whole course of pregnancy and quality of the pig litters.

The field observations seem to confirm the observations of the course of pregnancy on farms (M. Porowski, Vet-Com, personal communication) and in gilts after embryo transfer (Z. Smorag National Research Institute of Animal Production, Balice, personal communication), that frequent reason for litter losses could be the premature regression of corpora lutea, since gilts after recognizing the lack of pregnancy between day 40 and 50 express signs of estrus. Such situation is not possible in the presence of progesterone in blood [Webel *at al.* 1975] and it can be supposed, that the major cause of embryo losses in these cases was the regression of corpora lutea around day 30 of the pregnancy. Another evidence in favor of corpora lutea dysfunction in this period is the fact that luteal function was continued in the absence of living fetuses until day 60 of pregnancy, while all fetuses died on day 30 of pregnancy [Webel *at al.* 1975]. It may indicate that no intrauterine stimulus is needed to maintain the corpora lutea after day 30 of the pregnancy.

The above data correlate with the events during the pseudopregnancy in pigs, which is caused by the injections of pharmatological doses of estrogens between days 11-15 of the estrous cycle. The corpora lutea maintained in this way beyond the period of natural regression (days 14-17 of the estrous cycle) function even over 50 days [Pusateri *et al.* 1996]. Our concept of application of antiluteolytic agent relied on supporting the luteal function in two crucial periods, during the maternal recognition of pregnancy (days 11-13) and during peri- and post-implantation period (days 16-25 of pregnancy).

Earlier studies have already shown that a single hCG application on day 12 of pregnancy had a favorable effect on corpus luteum function and embryo survival until day 30 of pregnancy [Tilton *et al*.1989, Bolzan *et al.* 2013].

Our "semi-field" observations performed during the pregnancy and until weaning indicate rather that the hCG administration on day 20 of the pregnancy can be more beneficial for eventual limiting embryo mortality in pigs. Also the intravaginal estradiol and PGE_2 application on days 17-23 of pregnancy seems to be promising approach to improve embryo survival in pigs. However, taking into consideration the variability in responses and limited number of animals treated, it is unlikely that a practical treatment protocol can be developed at this time for the use on the commercial farms in order to improve embryonic survival and litter size without extending the studies on large number of animals and farms.

Acknowledgements. *We are grateful to Mrs. Katarzyna Gromadzka-Hliwa, Mrs. Joanna Murawska-Kempa and Mr. Jan Klos for technical assistance in the laboratory.*

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