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The Effect of Vaccination on Hematological Profiles of Piglets Born to Sows Injected with Gonadotropin Prior to Mating

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Abstract

The present experiment was designed to evaluate the hematological profiles of piglets born to sows injected with gonadotropin prior to mating and to study the effects of vaccination with hog cholera vaccine on the hematological profiles. At the age of 4 weeks, forty piglets were selected to study the effect of vaccination on hematological profiles of the piglets born to control sows injected with 0.90% NaCl prior to mating (20 piglets) and those born to sows injected with PMSG and hCG prior to mating (20 piglets). Blood samples were taken prior to vaccination and 4 weeks after vaccination to measure hematological parameters.

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Before vaccination, the erythrocyte, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hematocrit, and thrombocyte in piglets born to control sows and those born to sows injected with PMSG and hCG prior to mating were similar and were in normal ranges both prior to and after vaccination. However, both before vaccination and 4 weeks after vaccination, the leucocyte concentrations of the piglets born to sows injected with PMSG and hCG prior to mating were lower ($P<0.05$) by 28.04 and 28.82%, respectively, compared to piglets born to control sows. Both in piglets born to control sows without PMSG and hCG injection and those born to sows injected with PMSG and hCG prior to mating had higher leukocyte concentrations before vaccination and decreased ($P<0.05$) by 15.95 and 16.87%, respectively, 4 weeks after vaccination. It was concluded that the piglets born to sows injected with gonadotropin prior to mating had normal hematological profiles to support the greater postnatal growth. The lower leukocyte concentrations in the present experiment could be related to better health status and resistance to parasite that eventually decreased mortality in offspring born to sows injected with gonadotropin prior to mating.

Keywords: gilts; sows; leukocyte; hematological parameters; PMSG-hCG.

1. Introduction

The growth and development of embryos and fetuses during prenatal growth is affected and supported by the optimum uterine environment that provides nutrients, oxygen, and compounds required by the growing fetus to reach the optimum birth weight at parturition. The optimum growth and development of uterus and placenta is controlled and regulated by the pregnant hormones, especially estrogen and progesterone, as well as growth factors. Injection of the sows with gonadotropin prior to mating significantly increased pregnant hormones secretion [1].

Studies in swine, with the polytocus traits, with the litter sizes that can reach 18, showed that the improved endogenous secretions of pregnant hormones significantly improved the growth and development of uterus, placenta, embryo, and fetus [1], and improved birth weights and the pre-weaning growth of the offspring [2, 3, 4], and maternal milk production [5]. The final results were lower mortality rate, better growth of piglets until weaning and finishing weight with a better meat quality [5]. In addition, the pigs born to sows injected with PMSG and hCG prior to mating have higher and better growth rates so that they can reach the final body weight of 95 kg two weeks faster than those born to control sows.

In addition, sows injected with gonadotropin prior to mating had higher maternal serum thyroxin concentrations during pregnancy [6] that are supposed to contribute to the improved prenatal growth of the offspring. The improved prenatal and postnatal growth of the offspring was confirmed to be associated with the improved expression of growth hormone gene [7]. Preliminary study in sheep showed that infection of the lambs born to ewes injected with gonadotropin prior to mating showed a significant resistance and resilience with a positive growth rate during the infection while the lambs born to control ewes showed a decreased body weight during infection [8]. The improved prenatal and postnatal growths of the offspring born to gonadotropin-injected sows indicated a better organogenesis, including organogenesis of hematopoietic organs that support optimum hematopoiesis [9]. Optimum growth and health of individual animal is supported by the hematological profiles

to support optimum metabolism and growth rate. The health and survival of the individual animal is supported by the optimum body defense and immune systems. The present experiment was designed to study the effects of vaccination on hematological profiles of the post-weaned piglets born to control sows without gonadotropin injection and sows injected with PMSG and hCG prior to mating. The limitation of the present experiment was the unmeasured immunoglobulin and differential leukocyte profiles of the experimental piglets. In addition, the specific responses of the experimental piglets to hog cholera vaccine were not measured due to the technical limitation. In the future experiment, the profiles of these parameters in experimental animals born to gonadotropin-injected maternal animals should be measured.

2. Materials and Methods

The experiment was conducted in a commercial pig farm in Kelurahan Wailan, Kecamatan Tomohon Utara, Tomohon, North Sulawesi Province. The analysis of hematological profiles was conducted in the commercial laboratory in Manado, North Sulawesi Province.

2.1. Animal Maintenance

The experimental animals used were thirty mature female Landrace sows with body weight ranges of 95 – 105 kg. The experimental sows were fed with commercial ration used in the farm. The experimental sows were maintained in individual pens and were fed two times a days in the morning (07.00 WITA) and in the afternoon (15.00 WITA), and drinking water was available *ad libitum*. The experimental sows were injected with pregnant mare serum gonadotropin (PMSG) and human chorionic gonadotropin (hCG) (PG 600, Intervet, Netherlands) at a dose of 600 IU per sow. To synchronize estrous cycle, the experimental sows were injected with Prostaglandin ($PG_{2\alpha}$) (Lutalyse, Intervet, Netherlands) 2 times with 14 days interval. At the second $PG_{2\alpha}$ injection, or 3 days prior to estrus, 15 experimental sows were injected with PMSG and hCG intramuscularly, and the other 15 experimental sows were injected with 0.90% NaCl solution as a control. After showing estrus signs (red vagina, vaginal mucosa, and when the back was pressed the female sows were stand statically) the experimental sows were mated naturally by mixing them with selected boars. At the estrus period, the experimental sows were mated two times, one in the morning and repeated again in the afternoon.

2.2. Experimental Design

The experimental sows were assigned into a completely randomized design with 2 treatments. The first group of experimental sows was injected intramuscularly with physiological NaCl solution (0.90%) as a control. The second group of experimental sows was injected with PMSG and hCG intramuscularly. Each treatment used 15 experimental sows. Forty piglets born to the experimental sows (20 piglets from control sows injected with 0.90% NaCl and 20 piglets from sows injected with PMSG and hCG prior to mating) were used to measure blood parameters before and after vaccination. At the age of 4 weeks or one week after weaning, the experimental piglets were vaccinated with hog cholera vaccine. Prior to vaccination and four weeks after vaccination, the blood samples were collected to measure the hematological profiles of the experimental piglets after one month vaccination.

2.3. Sampling and Measurement of Parameters

The pregnant experimental sows were maintained individually until parturition. During parturition, the offspring born were identified by numbering. Blood samples were collected two times. The first blood samplings were conducted prior to vaccination of the experimental piglets at the age of 4 weeks. The second blood samplings were conducted 4 weeks after vaccination or at the age of 2 months. Blood samples were drawn from jugular vein and collected blood samples were stored in a sterile tube containing EDTA as an anticoagulant. One milliliter of blood sample was used to analyze blood parameters. Blood samples were collected in the morning at 09.00–11.00. The collected samples were analyzed in 24 hours period. The hematological parameters were measured by using Serono-Baker Diagnostic 9000 + (Inc Cascade Drive, Allentown, Pennsylvania 18103, USA). Parameters measured were the number of erythrocyte, hemoglobin concentration, hematocrit value, indexes of erythrocytes values i.e., mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), thrombocyte concentration, and total leucocyte concentration.

2.4. Data Analysis

The collected data were analyzed by using Analyses of Variance by using SPSS 20 software.

3. Results

The results of observation at the time of weaning (before vaccination) showed that the offspring born to maternal experimental sows injected with PMSG and hCG prior to mating showed better health and growth rate compared to those born to control sows without gonadotropin injection. As an indication of animal physiological status, total erythrocytes of the weaned piglets born to experimental sows injected with PMSG and hCG prior to mating tended to be higher by 0.28 x 10^6/ul (P>0.05) compared to those of weaned piglets born to control sows injected with physiological NaCl (Table 1). However, the erythrocyte concentration was still in the normal range. Four weeks after vaccination, total erythrocytes of weaned piglets born to sows injected with PMSG and hCG prior to mating tended to be higher by 0.67 x 10^6 /µL (P>0.05) compared to those of weaned piglets born to sows injected with physiological 0.9% NaCl (Table 1). The erythrocytes parameters in experimental piglets were not different before and after vaccination both in piglets born to control sows and sows injected with gonadotropin prior to mating. The hemoglobin parameters also tended to increase in the piglets born to sows injected with PMSG and hCG prior to mating compared to those in piglets born to control sows injected with physiological NaCl (P>0.05). Before vaccination, piglet born to sows injected with PMSG and hCG prior to mating tended to have higher hemoglobin concentration by 0.51 g/dL (P>0.05) compared to piglets born to control sows injected with physiological NaCl (Table 1). Four weeks after being vaccinated, the piglets born to sows injected with PMSG and hCG prior to mating tended to have higher hemoglobin concentration by 0.47 g/dL (P>0.05) compared to those unvaccinated piglets born to control sows injected with physiological NaCl prior to mating (Table 1). The hemoglobin concentrations of the experimental piglets born to control sows injected with physiological NaCl and sows injected with PMSG and hCG prior to mating both before and 4 weeks after vaccination were not different and still in the normal ranges. Based on statistical

analysis, both before vaccination and four weeks after vaccination, there was no significant difference (P>0.05) in hematocrit percentage of piglets born to sows injected with PMSG and hCG prior to mating compared to those born to control sows injected with physiological NaCl prior to mating. The average values of hematocrit percentage in piglets born to sows injected with PMSG and hCG prior to mating were numerically higher by 2% (P>0.05) compared to control piglets (Table 1). Four weeks after vaccination, the average value of hematocrit percentage in piglets born to sows injected with PMSG and hCG prior to mating tended to be higher by 1.5% (P>0.05) compared to control piglets (Table1). Vaccination did not affect hematocrit percentage in experimental piglets. The higher hematocrit value indicates the increase in blood viscosity due to the increased number of erythrocytes. Both before vaccination and four weeks after vaccination, the same pattern was also observed in mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hematocrit (MCHC) in the experimental piglets born to sows injected with PMSG and hCG prior to mating were similar to piglets born to control sows without PMSG and hCG injection (P>0.05). However, piglets born to sows injected with gonadotropin tended to have lower MCV (P>0.05) both before vaccination and four weeks after vaccination compared to control piglets (Table 1).

Table 1: Hematological profiles of piglets born to control sows injected with 0.90% NaCl and those born to sows injected with PMSG and hCG prior to mating at the age of 4 weeks postpartum prior to vaccination and 4 weeks after vaccination with hog cholera vaccine.

Parameters		Piglets born to sows injected with	
		NaCl 0.90%	PMSG-hCG
Erythrocyte (x 10^6/µL)	Before vaccination	5.67 ± 0.117	5.95 ± 0.204
	After vaccination	5.33 ± 0.516	6.00 ± 0.258
Hemoglobin (g/dL)	Before vaccination	9.53 ± 0.196	10.04 ± 0.359
	After vaccination	9.50 ± 0.342	10.17 ± 0.307
Hematocrit (%)	Before vaccination	34.08 ± 0.624	36.08 ± 0.124
	After vaccination	34.33 ± 0.760	35.83 ± 0.749
Mean Corpuscular Volume (MCV) (fL)	Before vaccination	60.12 ± 1.193	55.33 ± 3.715
	After vaccination	64.17 ± 2.023	59.00 ± 1.949
Mean Corpuscular Hemoglobin (MCH) (pg)	Before vaccination	16.84 ± 0.384	16.89 ± 0.325
	After vaccination	17.50 ± 0.428	16.67 ± 0.422
Mean Corpuscular Hematocrit (MCHC) (g/dL)	Before vaccination	27.95 ± 0.289	27.75 ± 0.347
	After vaccination	27.17 ± 0.477	27.83 ± 0.477
Thrombocyte (/µL)	Before vaccination	470.36 ± 72.191	549.27 ± 64.336
	After vaccination	508.00 ± 75.064	515.83 ± 91.409
Leukocyte (x 10^3/µL)	Before vaccination	36.49 ± 1.471[ac]	26.26 ± 1.206[bc]
	After vaccination	30.67 ± 3.730[ad]	21.83 ± 0.654[bd]

[a-d] Different superscripts in the same row indicate a significant difference (P<0.05).

Total thrombocyte of all experimental piglets both before and after vaccination and both in the offspring born to control sows and those born to sows injected with PMSG and hCG prior to mating were similar (P>0.05). Total thrombocytes in piglets born to sows injected with PMSG and hCG prior to mating tended to be higher compared to those born to control sows without PMSG and hCG injection prior to mating. However, in control piglets, there was a tendency of increased total thrombocyte after vaccination. In contrast, piglets born to sows injected with gonadotropin tended to have lower total thrombocyte after vaccination.

The most significant change in hematological profiles was observed in leukocyte parameter. Both before and 4 weeks after vaccination, the piglets born to sows injected with PMSG and hCG prior to mating had significantly lower total leukocytes concentrations (P<0.05) compared to those piglets born to control sows. Prior to vaccination, the averages of total leukocyte leukocyte concentration of piglets born to sows injected with PMSG and hCG prior to mating was lower by 10.23 x 10^3/µL compared to average total leukocyte concentration in piglets born to control sows injected with 0.90% NaCl prior to mating. After vaccination, the averages of total leukocyte concentration in piglets born to sows injected with PMSG and hCG prior to mating was lower by 8.84 x 10^3/µL (P<0.05) compared to piglets born to control sows without PMSG and hCG injection prior to mating. Four weeks after vaccination, the total leukocyte concentration of the experimental piglets decreased significantly (P<0.05). Control piglets born to control sows without gonadotropin injection prior to mating had 5.82 x 10^3/µL lower total leukocyte concentration (P<0.05) 4 weeks after vaccination. Piglets born to sows injected with PMSG and hCG prior to mating 4.43 x 10^3/µL lower total leukocyte concentration (P<0.05) 4 weeks after vaccination.

4. Discussion

The hematological profiles of the experimental piglets both born to control sows and those born to sows injected with gonadotropin prior to mating were in normal ranges. The most significant changes were observed in total leukocyte concentrations. In general, piglets born to sows injected with gonadotropin prior to mating, both before and after vaccination, had lower total leukocyte concentrations compared to control piglets born to control sows without gonadotropin injection. In addition, vaccination of experimental piglets with hog cholera vaccine significantly decreased total leukocyte concentration both in control piglets and those born to sows injected with gonadotropin. Since the growth performance and survival rates of offspring born to gonadotropin injected sows were consistently higher [3, 4, 5], the immune systems of the piglets born to gonadotropin-injected sows should be better. The observation in this reported experiment showed a lower mortality rate of the piglets born to gonadotropin-injected sows (unpublished observation). Low mortality rates were supported by the optimum immune systems.

It was assumed that piglets born to control sows without PMSG and hCG injection prior to mating had higher stress conditions. This assumption is linear with the results reported previously [9] that in the stress animals, total number of leukocytes in the peripheral were higher. Preliminary results in sheep showed that lambs born to gonadotropin-injected ewes showed a low infection conditions as was indicated by the higher resistance and resilience to *Haemonchus contortus* [8].

Even though it was not statistically significant, there is a different pattern of MCV and thrombocyte profiles. The MCV in piglets born to sows injected with PMSG and hCG prior to mating tended to be lower while vaccination tended to increase MCV in experimental gilts. Thrombocyte concentrations in piglets born to sows injected with PMSG and hCG tended to increase, but vaccination tended to increase thrombocyte in control piglets but decreased in piglets born to gonadotropin-injected sows. Again, these phenomena should have positive contribution to the improved prenatal and postnatal growths and higher survival rates of the gilts born to gonadotropin-injected sows [3, 4, 5].

Since blood cells and plasma components in blood system have very important functions in transport of nutrients and oxygen and carbon dioxide, regulatory, protective, and homeostatic functions [10] and hematological profiles reflects the physiological responsiveness of the animals to its internal and external environment [11], the hematological profiles observed in the piglets born to gonadotropin-injected sows were optimum to support body functions. According to the standard values for hematological parameters in pigs [12], all parameters observed in this experiment were in the normal ranges, except hemoglobin concentration and hematocrit in the offspring born to control sows that were slightly below the bottom limits.

Since most of the improved growth and development of offspring observed in offspring born to maternal animals injected with gonadotropin are occurred during organogenesis during pregnancy, further studies on the growth and development of organs related to hematological parameters are required. Preliminary results showed that piglets born to sows injected with gonadotropin prior to mating had greater organ weights compared to control piglets born to control sows without gonadotropin injection [9].

How does the treatment during pregnancy improve health status of the offspring during postnatal life? Improved endogenous secretions of pregnant hormones by injecting the sows with gonadotropin significantly improved the growth and development of uterus, placenta, embryo, and fetus [1], and improved birth weights and the pre-weaning growth of the offspring [2, 3, 4]. The improved uterine and placental environments in sows injected with gonadotropin will improve prenatal growth that will affect genetic expression [7] and organogenesis [9] that eventually affects the postnatal growth. The condition of uterus and placenta during prenatal growth will determine the genetic expression and growth and development of the embryo and fetus [13, 14, 15] that eventually determine the postnatal growth and health performances [16, 17, 18, 19]. The preliminary results of the present experiment clearly confirmed that the health status of the piglets born to sows injected with gonadotropin prior to mating was improved due to the improved organogenesis during prenatal life.

The health condition of an individual animal is strongly determined and affected by the growth and development during the prenatal life [19, 20, 21, 22]. The growth retardation due to the limited availabilities of nutrients required for growth and development will affect the health status during postnatal life [16, 17]. Therefore, in general, it is clear that postnatal growth and health performances of the offspring are programmed by the prenatal environment and conditions [23, 24]. Therefore, improvement of uterine and placental environment during prenatal growth by improving the endogenous secretions of pregnant hormones during pregnancy will improve the prenatal and postnatal growth of the offspring that will produce superior offspring with a better

growth rate and health performances. Our previous studies [1, 2, 3, 4, 5, 6, 7, 8, 9] strongly support this hypothesis and improved uterine and placental environment by optimizing the endogenous secretions of pregnant hormones during pregnancy by injecting the mother with gonadotropin prior to mating can be promoted to be applied in producing superior offspring.

5. Conclusion and Recommendation

The improved prenatal growth in piglets born to sows injected with gonadotropin prior to mating improve hematological parameters with a stable erythrocyte and thrombocyte profiles but lower total leukocytes that eventually will contribute to the healthier offspring as was indicated with the lower mortality and morbidity. This simple epigenetic technology could be applied in improving the growth and development of offspring during prenatal growth that eventually improves the postnatal growth as well as the health status and survival of the offspring. It is recommended that this simple technology can be used to produce superior offspring with better growth and health performances.

6. Conflict of Interest

This experiment has no conflict of interest.

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