Effects of Uteroferrin on Hematopoietic Parameters in Normal Pigs

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Abstract

Uteroferrin (UF), a progesterone-induced acid phosphatase from pig uterus (also called Type 5 tartrate-resistant acid phosphatase) and a secretory product of human placenta (human UF, hUF), is responsible for transplacental iron transport during early pregnancy in pigs. In addition, recent studies suggest an *in vitro* effect on hematopoietic progenitor cells. The present study examined the effects of UF on hematopoietic progenitor cells in bone marrow and peripheral blood in weaned pigs *in vivo*. UF injected i.m. (100 μg/kg twice daily) increased ($P< 0.05$) total white blood cell count on days 14-19 of the treatment period. UF increased circulating BFU-E (60%) and CFU-GM (37%) progenitor cells 8 hours after injection. The effect of 5-FU on young pigs was examined and a cumulative dose of 25 to 50 mg/kg was found to be optimal for myelosuppression. These results suggest that UF can stimulate circulating levels of hematopoietic progenitor cells *in vivo*, resulting in a potential increase in white and red blood cell numbers. Additional studies including interactions with other lineage specific hematopoietic growth factors, e.g. GM-CSF, will be necessary to confirm these results.

Introduction

Uteroferrin (UF) is a progesterone-induced acid phosphatase from pig uterus (also called Type 5 tartrate-resistant acid phosphatase) (Bazer et al., 1984), and a secretory product of human placenta (human UF, hUF) (Ketcham et al., 1985). UF is responsible for transplacental iron transport during early pregnancy in pigs (Roberts et al., 1986). Recently, *in vitro* studies suggest that UF may also be a hematopoietic growth factor. UF has been demonstrated to have colony forming unit (CFU) activity *in vitro* for committed erythroid (BFU-E), granulocyte-monocyte/macrophage (CFU-GM), and granulocyte, erythrocyte, monocyte/macrophage, megakaryocyte cells (CFU-GEMM) (Bazer et al., 1991). Preliminary results also indicate that UF can affect hematopoiesis *in vivo* resulting in an increase in the numbers of BFU-E, CFU-GM, and CFU-GEMM committed cells present in bone marrow and peripheral blood (Edwards,

1990). In the present study, the effects of UF on hematopoietic progenitor cells in bone marrow and peripheral blood in weaned pigs were evaluated to determine if UF can affect hematopoietic progenitor cells *in vivo*.

Materials and Methods

Female pigs 14-22 kg were obtained from the Texas A&M University Swine Center. Initial animal weights were determined and animals allotted by weight and litter to treatment groups. Animals were housed in the Physiology and Metabolism Center at the Swine Unit, and penned by treatment.

Uteroferrin was purified from the uterine flushings of day 75 psuedopregnant gilts as described previously (Chen et al., 1973, Baumbach et al., 1986). Psuedopregnancy was induced by once daily injections of estradiol valerate (5 mg) on days 11- 15 of the cycle (Frank et al., 1977). On day 75, uteri were collected by midventral laparotomy and uterine horns flushed with 200 ml saline. Flushings were centrifuged (3000 rpm, 30 min, 4 C), and the resulting supernatant dialyzed using Spectra/Por molecularporous membrane (6000-8000 MW cutoff) tubing into 0.1 M Tris (pH 8.6). Dialyzed supernatant was applied onto a DEAE-52 anion exchange resin and uteroferrin eluted with 0.1 M Tris containing 0.5 M NaCl. The visible purple fraction was collected and concentrated using a diafiltration unit with a 10,000 MW cutoff membrane (Amicon Corporation, Beverly, MA). Concentrated UF was filtered (0.2 μm filter) to remove particulate material and applied onto a Sephacryl-100 column. Fractions containing UF were identified by absorbance at 545 nm and purity assessed by using SDS-PAGE. UF containing fractions were pooled and concentrated by diafiltration. Concentrated UF was desalted into 0.9% NaCl using a G-25 Sephadex Superfine gel filtration column. Concentration of UF was determined spectrophometrically using the absorbance at 545 nm based on an extinction coefficient of 3100 and a M.W. of 35,000 (Baumbach et al., 1986).

Experiment 1. Four littermate gilts (n=2/treatment) were treated with either UF (100 µg/kg) or an equivalent volume of 0.9% NaCl twice daily (0800 and 2000 h) for 21 days. On days 0, 7, 14, 21, and 28 CFU assays were conducted as outlined. Blood samples were obtained three times per week and total WBC, RBC, and thrombocytes determined and differential blood cell counts performed. Body weights were determined at weekly intervals and dosages adjusted accordingly.

Experiment 2. Experiment 1 was repeated with 12 pigs (n=6/treatment) treated with either UF (100 µg/kg) or an equivalent volume of 0.9% NaCl twice daily (0800 and 2000 h) for 19 days. Blood samples were obtained three times per week and total WBC, RBC, and thrombocytes determined and differential blood cell counts performed. Body weights were determined at weekly intervals and dosages adjusted accordingly.

Experiment 3. Mature pigs (100 kg) were assigned randomly to treatment groups (n=2/ treatment). Following initial blood collection, pigs were injected i.m. with UF (70 µg/kg in 0.9% NaCl) or an equivalent volume of 0.9% NaCl (control). Peripheral blood was collected again 8 hours post-injection. CFU assays were performed as outlined.

Experiment 4. This experiment examined the dose of 5-Flourouracil (5-FU) needed to attain myelosuppression in young swine. 5-FU was donated generously by Dr. Diana Worthington-White, University of Florida Medical School. 5-FU was reconstituted in 0.9% NaCl (pH 7.8) at a concentration of 41.7 mg/ml. Twelve pigs (n=3/treatment) were infused i.v. with one of four doses 5-FU (12.5, 25, 37.5, or 50 mg/kg respectively) on days 1 and 2. Blood counts were conducted as outlined, with the baseline obtained by averaging the results from three days prior to treatment.

Total Blood Cell Counts and Differential Counts. Blood samples (2 ml) were obtained by jugular venipuncture three times per week (Mon, Wed, and Fri) and analyzed by the Clinical Pathology Laboratory at the Texas A&M University, College of Veterinary Medicine. Baselines were established by averaging results for the three days prior to initiation of treatment.

Column Forming Unit Assays. Anesthesia was induced by injection of Telazol (44 µg/kg) and maintained with halothane. Bone marrow (5 ml) was collected aseptically into 20 ml syringes containing 1.0 ml heparin (1000 u/ml) using bone marrow biopsy needles. Mononuclear cells were isolated by layering 10 ml of Ficoll (Sigma, St. Louis, MO) under the blood and tubes centrifuged (30 min, 1500 RPM, 4 C). Interface cells were collected and washed twice with HBSS. Red blood cells were lysed osmotically by resuspension in 0.2% NaCl for 30 sec after which an equivalent volume of 1.6% NaCl was added, along with HBSS to 15 ml. Cells were pelleted (5 min, 1500 RPM, 4 C) and the supernatant aspirated. Cells were resuspended in RPMI + 20% FBS. Adherent cells were removed by incubating the cells in T75 tissue culture flasks (37 C) for 2 h. Non-adherent cells were collected and cell concentration determined using a hemocytometer. Cells were diluted to 4.0×10^6 cells/ml with RPMI + 20% FBS. CFU assays were performed in 0.3% agar. BFU-E assays were performed in IMDM + 1u/dish rhEPO (Sigma Chemical Company, St. Louis, MO). CFU-GM assays were plated in α-MEM + 100u/dish rhGM-CSF (R&D Systems, Inc, Minneapolis, MN). CFU-GEMM were plated in α-MEM, with both rhEPO (1u/dish) and rhGM-CSF (100u/dish) Colonies were counted on Day 14, where a colony is defined as a group of 50 or more cells.

Statistical Analysis. Data were analysed by the General Linear Model of SAS. The model included treatment, day and treatment x day, with gilt (treatment x day) as the appropriate error term.

Results

Experiment 1 analyzed the effects of UF on non-adherent bone marrow cell populations in normal pigs. Young pigs (initial weight 26.1 $\pm$ 0.5 kg) received twice daily i.m. injections of UF (100µg/kg in 0.9% NaCl) or an equivalent volume of 0.9% NaCl (control) on days 1 through 21 of the experimental period. Bone marrow was collected initially (measurement 1) and at 7 day intervals (measurements 2-5) during the experimental period. BFU-E colonies had initial means of 229 and 252 for UF and controls, respectively. By measurement 4, BFU-E colonies for both treated and control pigs had increased ($P< 0.05$) to 452 and 450 (Figure 1). UF increased ($P< 0.05$) BFU-E progenitor cells only on measurement 5 (425 vs. 316 for UF and control, respectively). While the control group was higher ($P< 0.05$) on measurement 2 (335 vs. 190). Baseline values for CFU-GM colonies were 73 and 38 for control and treated pigs, respectively, making the control group higher ($P< 0.05$) (Figure 2). By measurement 2, the UF treated group had increased to 49.3, while controls had dropped to 25.7. At measurement 5, there was also a difference ($P< 0.05$) with the UF group at 36 and the controls at 25. CFU-GEMM colonies showed an upward trend in the UF treated pigs from 24 initially to 34 by measurement 5, but the only difference ($P< 0.05$) was detected on measurement 5 (Figure 3).

For total and differential blood cell counts, no significant effect of experiment was indicated; hence data from Experiments 1 and 2 were pooled. The combined data from Experiments 1 and 2 were analyzed for effects of UF on total and differential cell counts in peripheral blood of normal pigs. Sixteen pigs (n=8/treatment) were injected with either UF (100µg/kg in 0.9% NaCl) or 0.9% NaCl i.m. twice daily for 19 days. No significant treatment effect was detected for any of the blood parameters measured ($P> 0.10$). White blood cell numbers in the treated group tended to increase, and by day 19 were higher ($P< 0.05$) than controls (Figure 4). Due to the difference in baseline values (19.3 for controls vs. 17.8 for treated), percentage changes from baseline were used to analyze these data (Figure 5). The percentage change in WBC count increased ($P< 0.10$) for the UF group on days 14 through 19

indicating a delayed response to UF. Red blood cell counts were not affected (P> 0.10) by treatment (Figure 6). Thrombocytes tended to decrease in the UF group (Figure 7), but not the control group (P> 0.10) The differential cell counts also failed to show a response to UF. Neutrophils were maintained near 35% of total WBC (Figure 8), and on day 10, were higher (P< 0.05) for the UF treated pigs. However, this was due to a decrease in the control group rather than to a real increase in the UF group. Lymphocyte numbers appeared to change inversely to that of neutrophils (Figure 9). On day 10, when the UF group was higher in neutrophils, control pigs had higher (P< 0.05) lymphocytes. Basophils increased from a baseline value of 0.25 to 1.0 by day 14 in the UF group, but fell below values for control pigs by day 19 (Figure 10). Eosinophils tended to be higher in the UF than the control pigs, but differences were not significant (Figure 11). Monocytes were not affected by treatment, as both groups varied concurrently from baseline values (Figure 12).

The acute infusion of UF into mature pigs (Experiment 3) appears to have affected progenitor cell levels in peripheral blood within 8 hours of injection. BFU-E levels increased (P< 0.05) from 132.6±16.1 to 197.8±11.0 in the UF pigs, while values for control pigs were unchanged (158.8±14.3 vs. 142.3±11.5; P>.05; Figure 13). CFU-GM levels increased (P< 0.05) in the UF pigs from 21.5±2.5 to 34.0±3.5, while values for control pigs did not change during the treatment period (P> 0.05) (17.1±2.0 vs. 21.4±2.5; Figure 14).

Experiment 4 examined the effects of 5-FU on total blood cell and differential cell counts in pigs. Limited information is available on the myelosuppressive effects of 5-FU in pigs. Dosages in the present study were based on results of Van Zant (1984), O'Reilly et al (1988) with mice, and the human dosage of 15 to 45 mg/kg (Stewart et al., 1993). All doses caused significant myelosuppression. The highest dose of 50 mg/kg resulted in 100% mortality between days 8 and 12 post infusion. At 37.7 mg/kg pigs experienced a 93% reduction in WBC by day 8

and had a 100% mortality rate between days 9 and 13. A 33% mortality rate was seen in the 25 mg/kg group (Figure 15) on day 13. This group experienced an 84% reduction in WBC count by day 8. Pigs receiving 12.5 mg/kg experienced a 33% suppression of total WBC counts by day 8, which recovered to 89% of initial values by day 13. This group also had an increase in monocytes relative to other types of white blood cells (Figure 16) and retained neutrophils through day 8 post infusion (Figure 17).

Discussion

In vivo experiments with hematopoietic growth factors (GM-CSF, G-CSF, IL-3, etc.) in a number of species have provided a framework for evaluating the results from the present experiments with UF. Gallicchio et al. (1989) infused mice with rmIL-1 and circulating WBC counts increased from 146% to 266% depending on dosage. This was largely attributed to an increase in neutrophils. Schuening et al. (1989) tested the effects of rhGM-CSF on canine hematopoiesis *in vivo*. Neutrophils, monocytes, and lymphocytes increased from three- to six-fold following infusion of rhGM-CSF, but returned to normal levels 3 to 7 days after injections were discontinued. While eosinophils, reticulocytes, and hematocrits were unchanged, platelet numbers decreased rapidly after infusions were initiated, and returned to normal within 24 hours after the last injection. Metcalf et al. (1987) found that rmGM-CSF increased circulating neutrophils two-fold, but had no significant effect on other peripheral blood cells. The *in vivo* effects of most hematopoietic growth factors are quite variable, making it difficult to conclude whether UF indeed has hematopoietic activity based strictly on the results derived from the present study. Some growth factors have marked effects on various cell lineages in peripheral blood, while others have little or no effect. Trends detected in the present study can be inferred to relate to the effects of other growth factors. It is possible that the decrease in platelets, while not significant, could be analogous to the decreases reported by Metcalf et al. (1987) when rhGM-CSF was tested in mice.

With these uncertainties in mind it is important to understand possible reasons why a dramatic treatment effect of Uf was not detected. The pigs could have metabolized the exogenous UF as an iron source. The bioavailability of iron from UF is greater than for any inorganic iron source making it difficult to eliminate this possibility from the experimental design. It is also possible that UF affects only primitive hematopoietic progenitor cells to increase their responsiveness to other growth factors for completion of the hematopoietic process. This may explain the delayed increase in total WBC numbers. Future experiments could be conducted in which another cytokine (GM-CSF for example) is injected simultaneously to test this hypothesis. The pig may also be so adept at maintaining homeostasis that the infusion of exogenous growth factors triggers a negative feedback pathway resulting in a decrease in the production of endogenous cytokines.

The increases in hematopoietic progenitor cell populations in peripheral blood at 8 h post-injection indicates that UF stimulates the release of progenitor cells into the blood stream or that UF stimulates the actual differentiation of pluripotent stem cells in the marrow. Consequently, *in vitro* responsiveness to EPO (Figure 13) and GM-CSF (Figure 14) is increased significantly. If UF is indeed stimulating either the release or differentiation of progenitor cells, why then do we not see a corresponding increase in those cells when UF is administered over an extended period. Perhaps the pigs has mechanisms to prevent the overproduction of blood cells and that mechanism is not yet understood. Using myelosuppressed pigs in future studies may yield further information on the presence of such feedback systems.

Experiment 4 has provided us with additional insight into the proper dose of 5-FU needed to simulate chemotherapy. Thus we can use the pig as a model for human cancer patients in future *in vivo* studies to determine if UF has potential as a therapeutic drug to enhance recovery of the hematopoietic system. A cumulative dose of 25-50 mg/kg will provide adequate myelosuppression while minimizing mortality.

Implications

The exact role of UF in hematopoiesis and any mechanisms by which it affects progenitor cell populations is still unclear. Further *in vivo* testing in conjunction with myelosuppression, and the use of GM-CSF should shed additional light on these effects and their mechanisms. It is still too early to determine if UF will indeed prove to have any therapeutic effects for human patients suffering from a variety of cancers or other immune system disorders.

Acknowledgments

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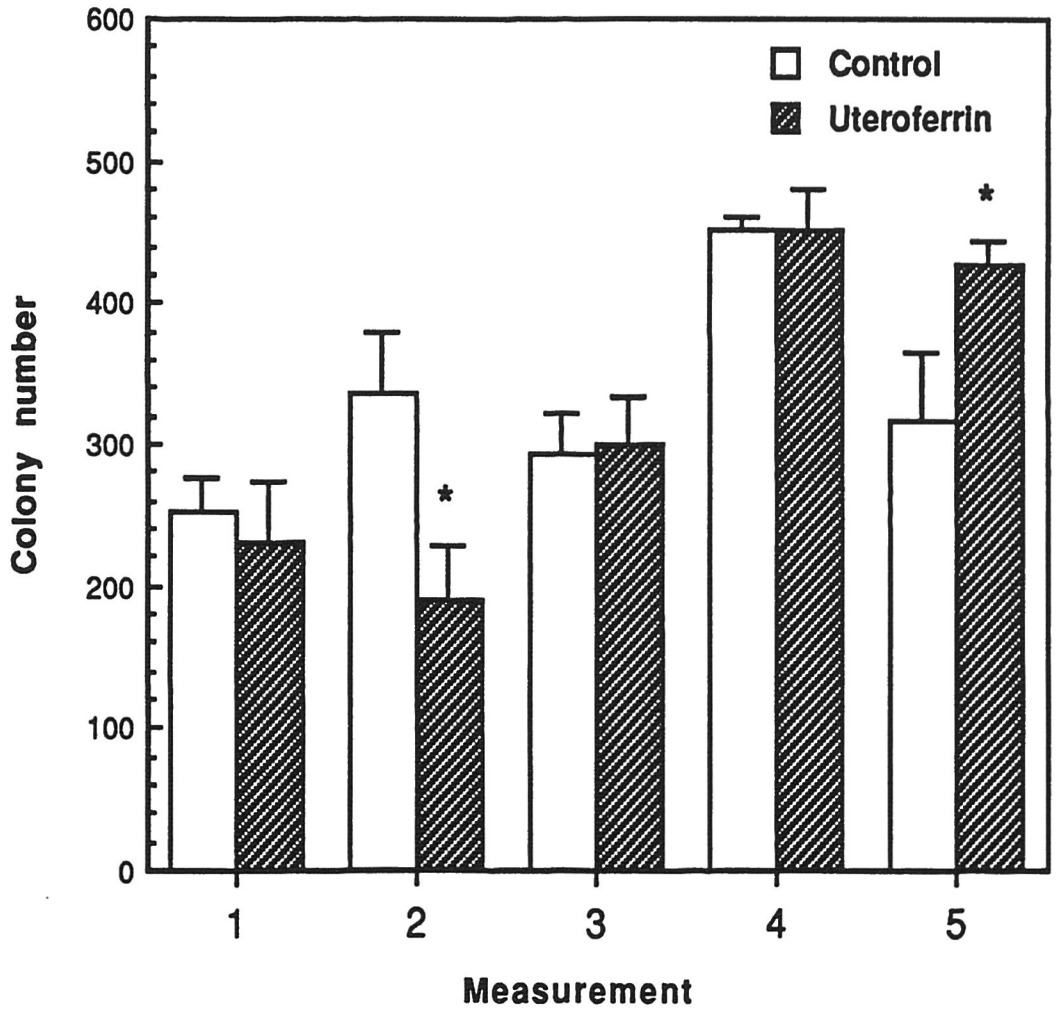

Figure 1. The effect of *in vivo* administration of uteroferrin on nondherent pig bone marrow cells when assayed for BFU-E progenitor cells. Young swine (initial weight 26.1± 0.5 kg) were assigned randomly to one of two treatment groups. Following measurement 1 (d=0) pigs received twice daily i.m. injections of uteroferrin (100 µg/kg in 0.9% NaCl) or an equivalent volume of 0.9% NaCl (control) on days 1 throgh 21 of the experimental period. Bone marrow was collected initially (measurement 1) and at 7 day intervals (measurements 2-5) during the experimental period. BFU-E assays were performed in 0.3% agar using 1 U rhEPO/dish as the stimulant. Values represent mean±SEM (n=2 animals/treatment). * Differs from control ($P < 0.05$).

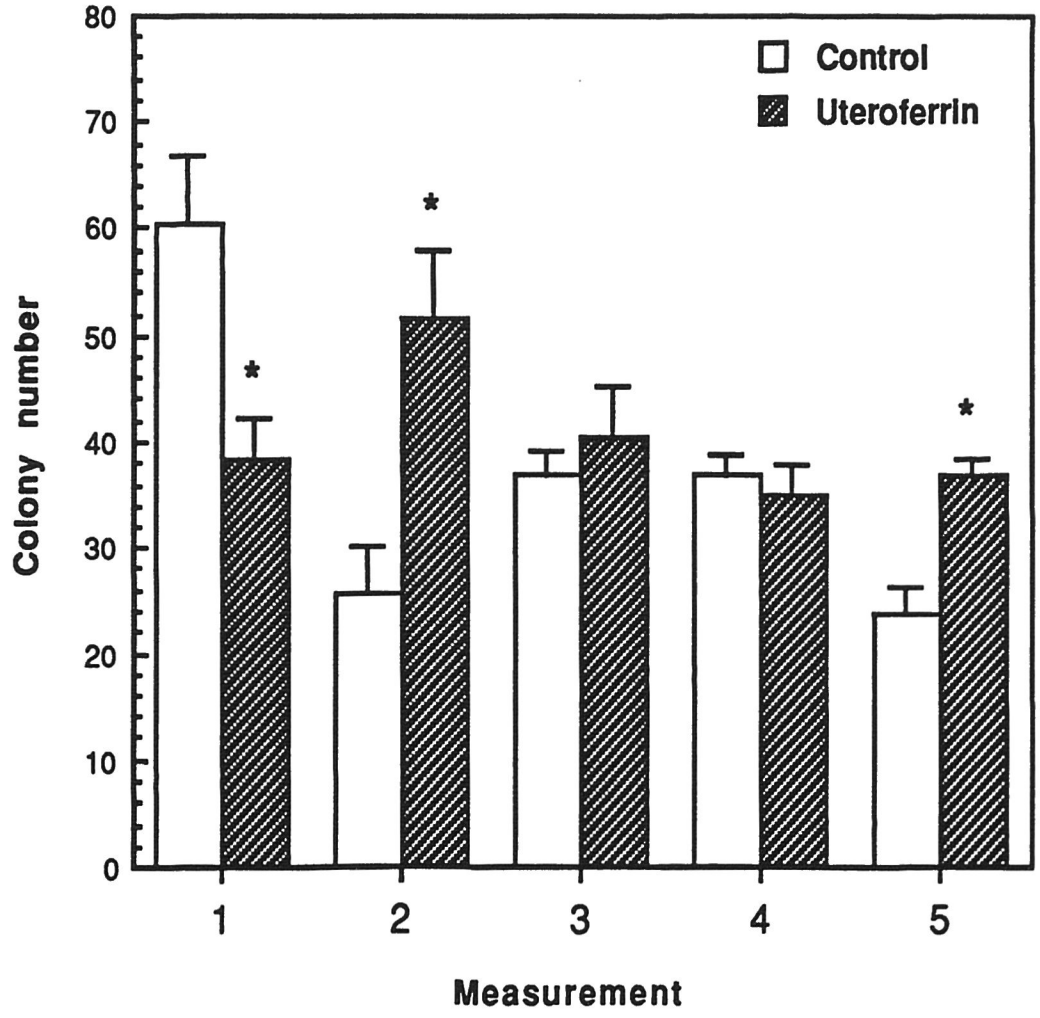

Figure 2. The effect of *in vivo* administration of uteroferrin on nondherent pig bone marrow cells when assayed for CFU-GM progenitor cells. Young swine (initial weight 26.1± 0.5 kg) were assigned randomly to one of two treatment groups. Following measurement 1 (d=0) pigs received twice daily i.m. injections of uteroferrin (100 µg/kg in 0.9% NaCl) or an equivalent volume of 0.9% NaCl (control) on days 1 throgh 21 of the experimental period. Bone marrow was collected initially (measurement 1) and at 7 day intervals (measurements 2-5) during the experimental period. CFU-GM assays were performed in 0.3% agar using 100 U rhGM-CSF/dish as the stimulant. Values represent mean±SEM (n=2 animals/treatment). * Differs from control ($P < 0.05$).

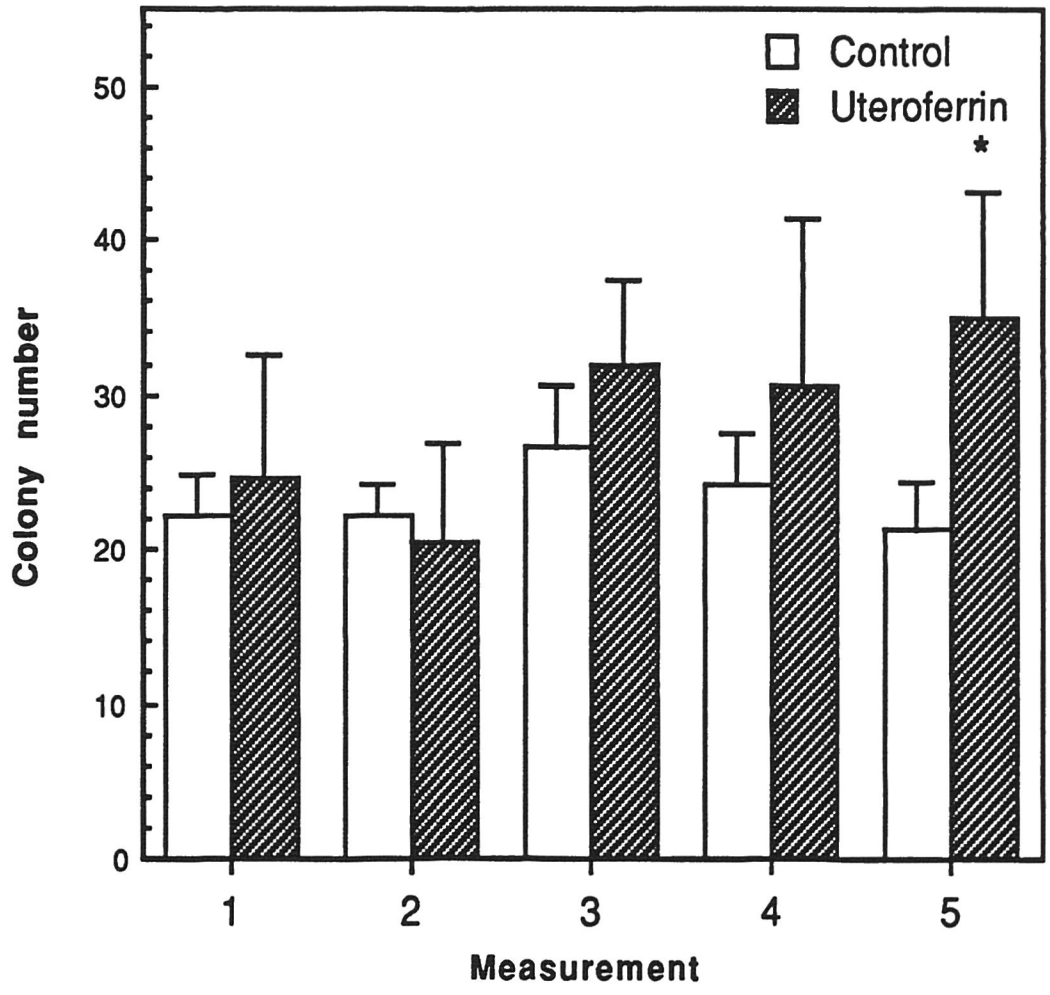

Figure 3. The effect of *in vivo* administration of uteroferrin on nondherent pig bone marrow cells when assayed for CFU-GEMM progenitor cells. Young swine (initial weight 26.1± 0.5 kg) were assigned randomly to one of two treatment groups. Following measurement 1 (d=0) pigs received twice daily i.m. injections of uteroferrin (100 μg/kg in 0.9% NaCl) or an equivalent volume of 0.9% NaCl (control) on days 1 throgh 21 of the experimental period. Bone marrow was collected initially (measurement 1) and at 7 day intervals (measurements 2-5) during the experimental period. CFU-GEMM assays were performed in 0.3% agar using 1 U rhEPO and 100 U rhGM-CSF/dish as the stimulant. Values represent mean±SEM (n=2 animals/treatment). * Differs from control ($P < 0.05$).

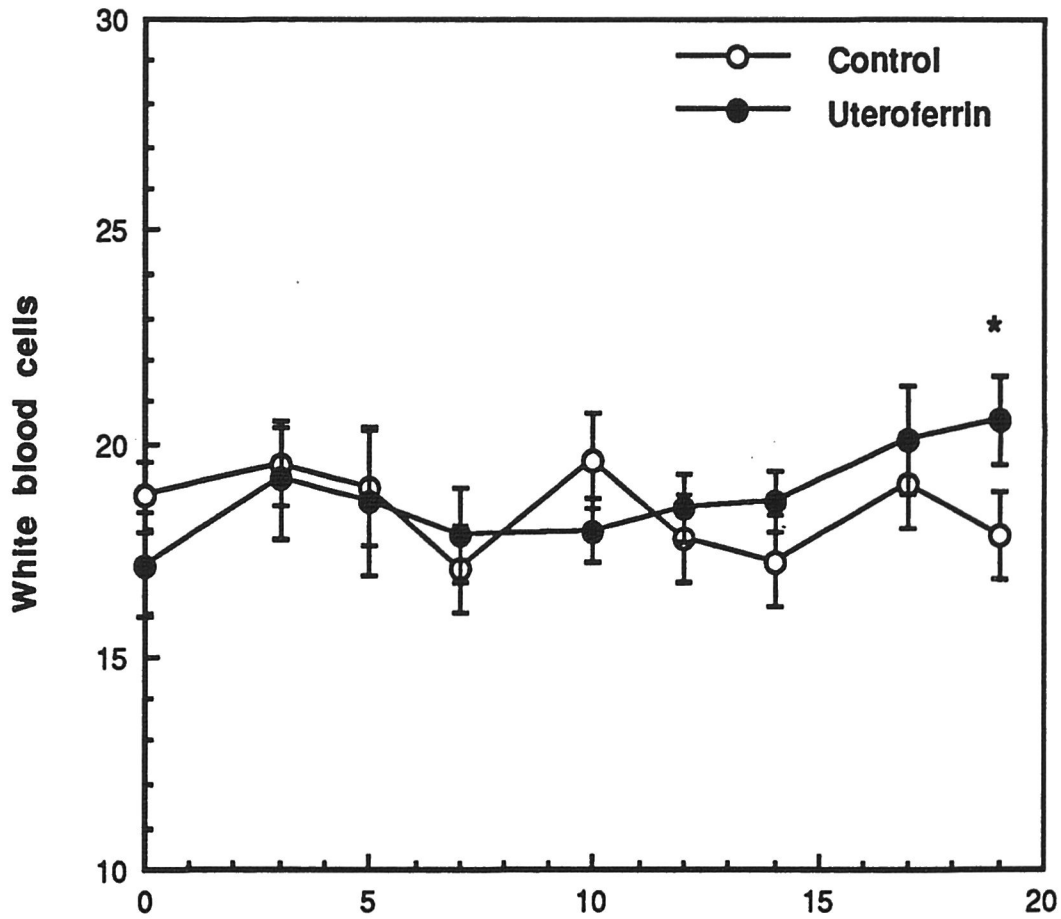

Figure 4. The effect of administration of uteroferrin on total white blood cell counts (cells x $10^3/\mu l$) in peripheral blood of young pigs. Pigs were assigned randomly to one of two treatment groups. Baseline counts were acheived via collection of peripheral blood on three consecutive days prior to the initiation of treatments. Beginning on day 1 and continuing throughout the experiments, pigs received twice daily i.m. injections of uteroferrin (100µg/kg in 0.9% NaCl) or an equivalent volume of 0.9% NaCl (control). Blood samples were taken three times/week and total white blood cell counts determined. Points represent means± SEM of two separate experiments (n=8 total animals/treatment/time period). *Differs from control ($P< 0.05$).

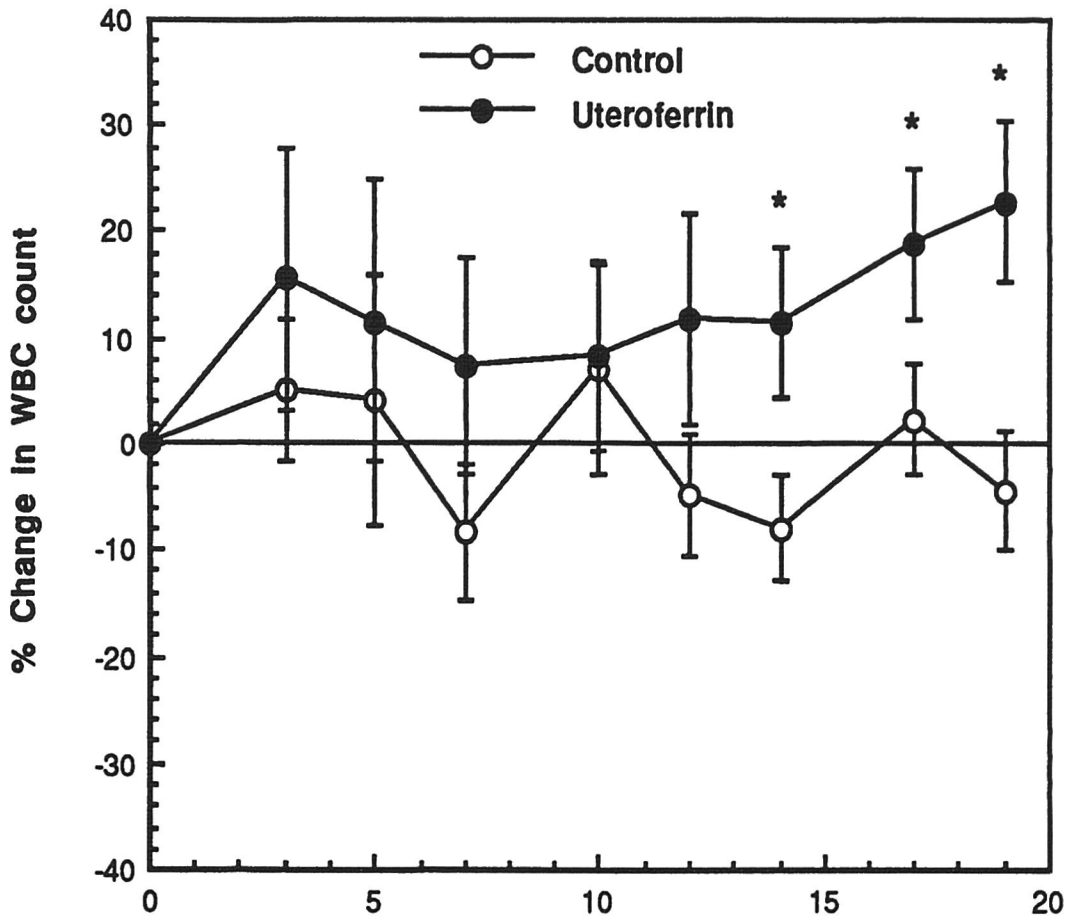

Figure 5. The effect of administration of uteroferrin on the percentage change in white blood cell counts in peripheral blood of young pigs. Pigs (initial weight 22.1 ± 1.5 kg) were assigned randomly to one of two treatment groups. Baseline counts were acheived via collection of peripheral blood on three consecutive days prior to the initiation of treatments (mean=d 0). Beginning on day 1 and throughout the course of the experiment, pigs received twice daily i.m. injections of uteroferrin (100 μg/kg in 0.9% NaCl) or an equivalent volume of 0.9% NaCl (control). Blood samples were taken three times/week and total white blood cell counts determined and the percentage change from baseline values calculated. Points represent means±SEM from two separate experiments (n=8 total animals/treatment/time period). *Differs from control ($P < 0.10$).

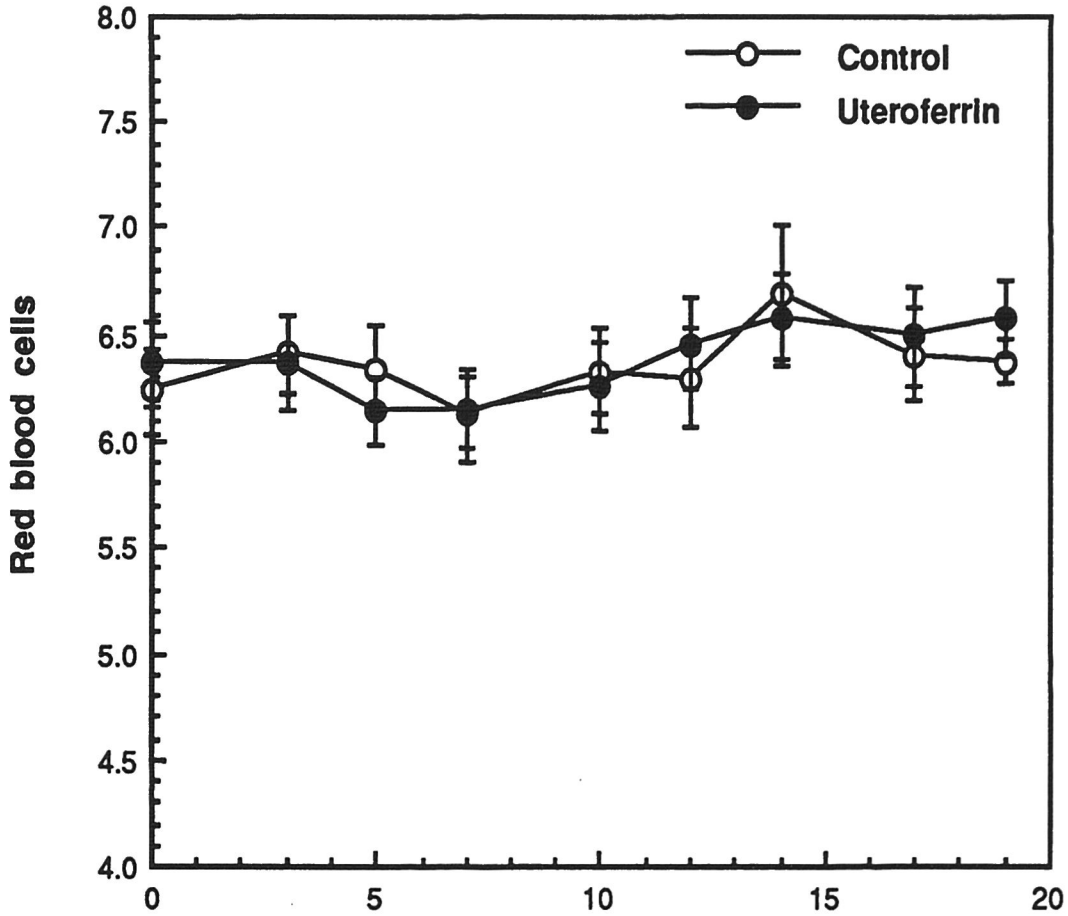

Figure 6. The effect of administration of uteroferrin on red blood cell counts (cells x $10^6/\mu l$) in peripheral blood of young pigs. Pigs (initial weight 22.1 ± 1.5 kg) were assigned randomly to one of two treatment groups. Baseline counts were acheived via collection of peripheral blood on three consecutive days prior to the initiation of treatments (mean=d 0). Beginning on day 1 and throughout the course of the experiment, pigs received twice daily i.m. injections of uteroferrin (100 μg/kg in 0.9% NaCl) or an equivalent volume of 0.9% NaCl (control). Blood samples were taken three times/week and red blood cell counts determined. Points represent means±SEM from two separate experiments (n=8 total animals/treatment/time period). No significant effect of treatment was detected ($P > 0.10$).

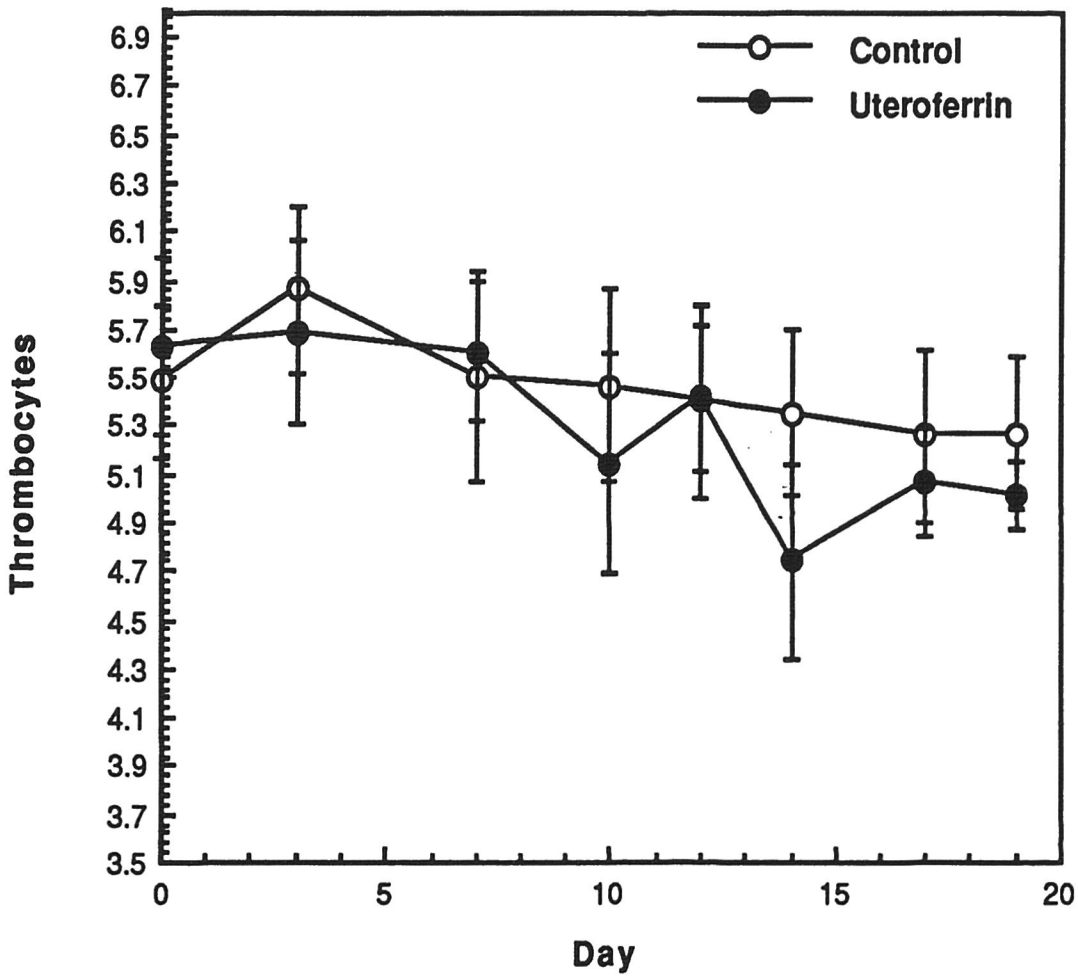

Figure 7. The effect of administration of uteroferrin on thrombocytes (cells x $10^5/\mu l$) in peripheral blood of young pigs. Pigs were assigned randomly to one of two treatment groups. Baseline counts were acheived via collection of peripheral blood on three consecutive days prior to the initiation of treatments. Beginning on day 1 and continuing throughout the experiments, pigs received twice daily i.m. injections of uteroferrin (100µg/kg in 0.9% NaCl) or an equivalent volume of 0.9% NaCl (control). Blood samples were taken three times/week and thrombocyte counts determined. Points represent means± SEM of two separate experiments (n=8 total animals/treatment/time period). No significant effect of treatment detected ($P > 0.10$).

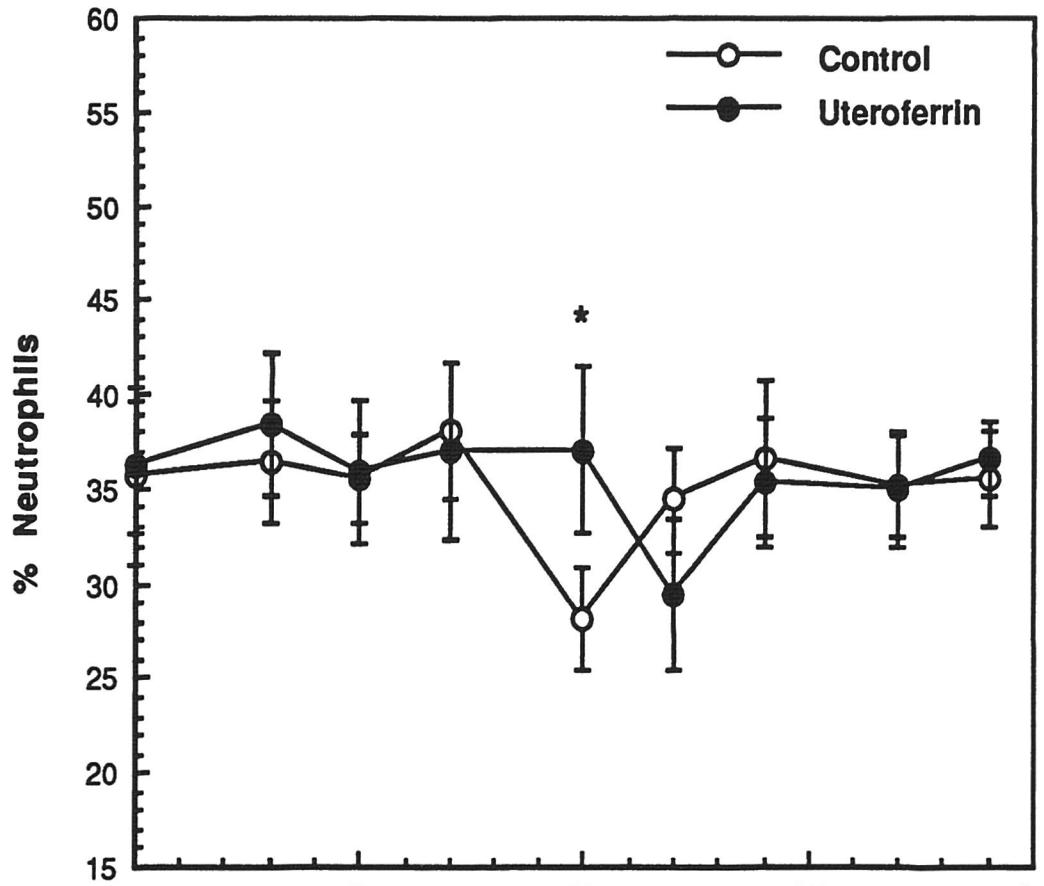

Figure 8. The effect of administration of uteroferrin on the percentage of neutrophils in white blood cell differential counts from peripheral blood of young pigs. Pigs (initial weight 22.1 ± 1.5 kg) were assigned randomly to one of two treatment groups. Baseline counts were acheived via collection of peripheral blood on three consecutive days prior to the initiation of treatments (mean=d 0). Beginning on day 1 and throughout the course of the experiment, pigs received twice daily i.m. injections of uteroferrin (100 μg/kg in 0.9% NaCl) or an equivalent volume of 0.9% NaCl (control). Blood samples were taken three times/week and percent neutrophils determined. Points represent means±SEM from two separate experiments (n=8 total animals/treatment/time period). *Differs from control ($P < 0.05$).

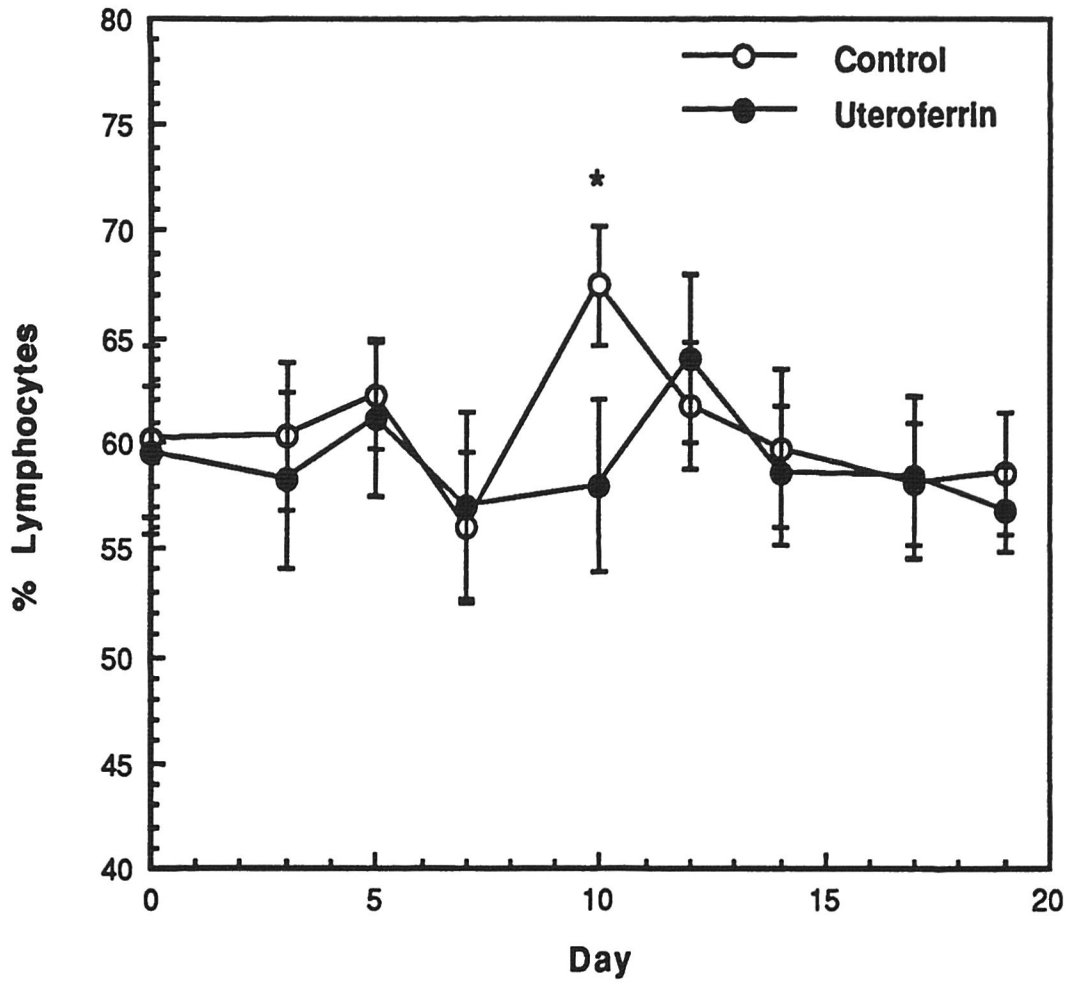

Figure 9. The effect of administration of uteroferrin on the percentage of lymphocytes in white blood cell differential counts from peripheral blood of young pigs. Pigs (initial weight 22.1 ± 1.5 kg) were assigned randomly to one of two treatment groups. Baseline counts were acheived via collection of peripheral blood on three consecutive days prior to the initiation of treatments (mean=d 0). Beginning on day 1 and throughout the course of the experiment, pigs received twice daily i.m. injections of uteroferrin (100 μg/kg in 0.9% NaCl) or an equivalent volume of 0.9% NaCl (control). Blood samples were taken three times/week and % lymphocytes determined. Points represent means±SEM from two separate experiments (n=8 total animals/treatment/time period). *Differs from control ($P < 0.05$).

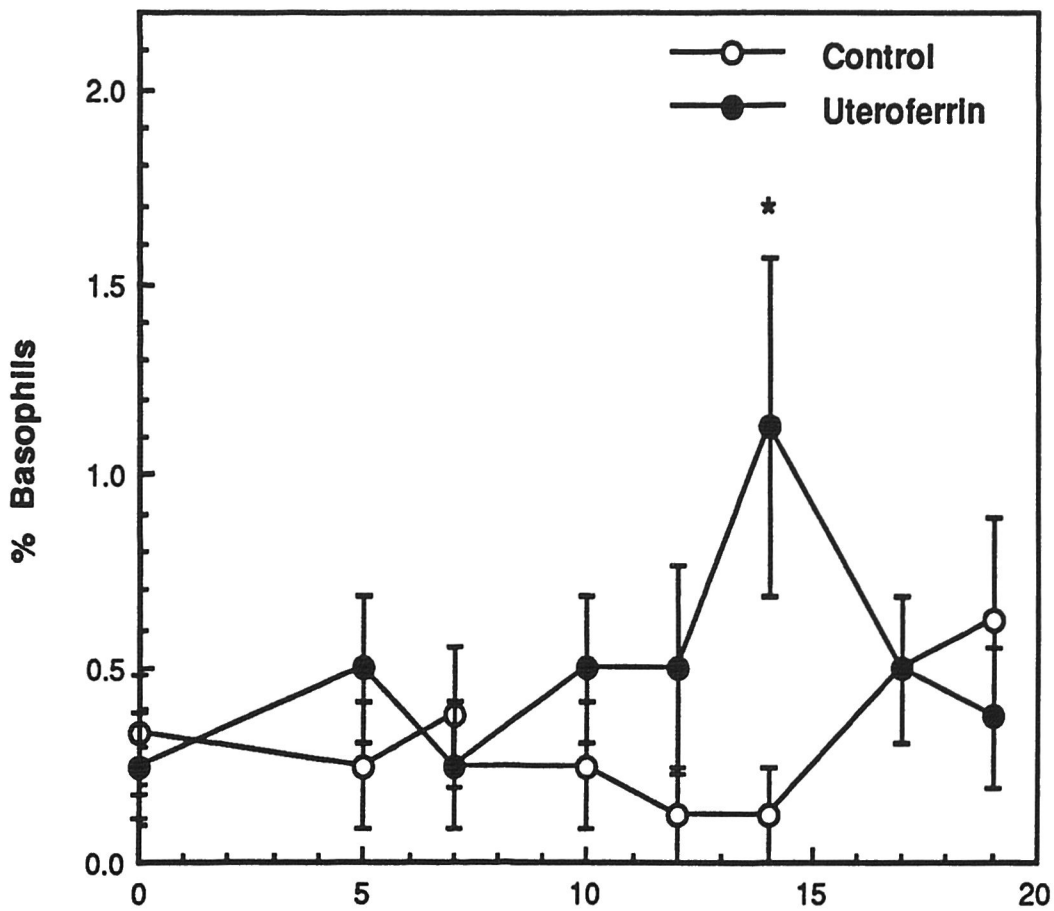

Figure 10. The effect of administration of uteroferrin on the percentage of basophils in white blood cell differential counts from peripheral blood of young pigs. Pigs (initial weight 22.1 ± 1.5 kg) were assigned randomly to one of two treatment groups. Baseline counts were acheived via collection of peripheral blood on three consecutive days prior to the initiation of treatments (mean=d 0). Beginning on day 1 and throughout the course of the experiment, pigs received twice daily i.m. injections of uteroferrin (100 μg/kg in 0.9% NaCl) or an equivalent volume of 0.9% NaCl (control). Blood samples were taken three times/week and percent basophils determined. Points represent means±SEM from two separate experiments (n=8 total animals/treatment/time period). *Differs from control ($P < 0.05$).

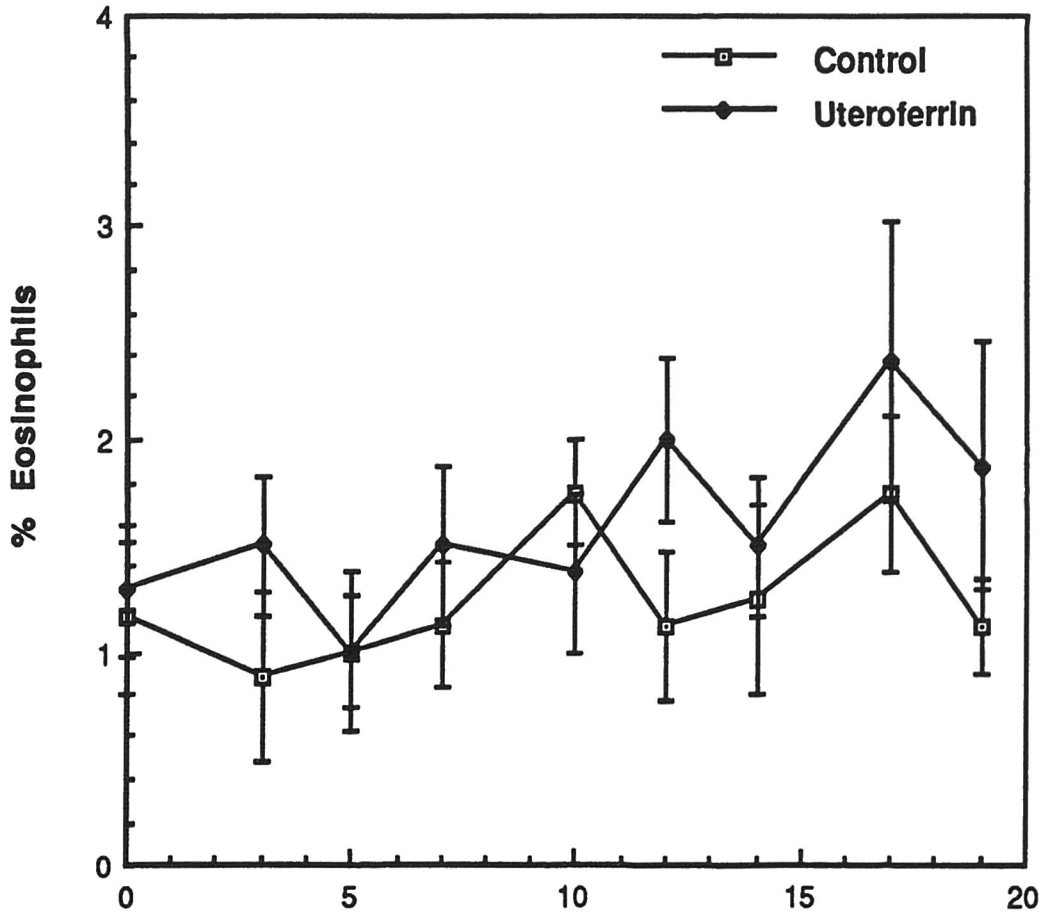

Figure 11. The effect of administration of uteroferrin on the percentage of eosinophils in white blood cell differential counts from peripheral blood of young pigs. Pigs (initial weight 22.1 ± 1.5 kg) were assigned randomly to one of two treatment groups. Baseline counts were acheived via collection of peripheral blood on three consecutive days prior to the initiation of treatments (mean=d 0). Beginning on day 1 and throughout the course of the experiment, pigs received twice daily i.m. injections of uteroferrin (100 μg/kg in 0.9% NaCl) or an equivalent volume of 0.9% NaCl (control). Blood samples were taken three times/week and % eosinophils determined. Points represent means±SEM from two separate experiments (n=8 total animals/treatment/time period). No significant differences due to treatment were observed (P> 0.10)

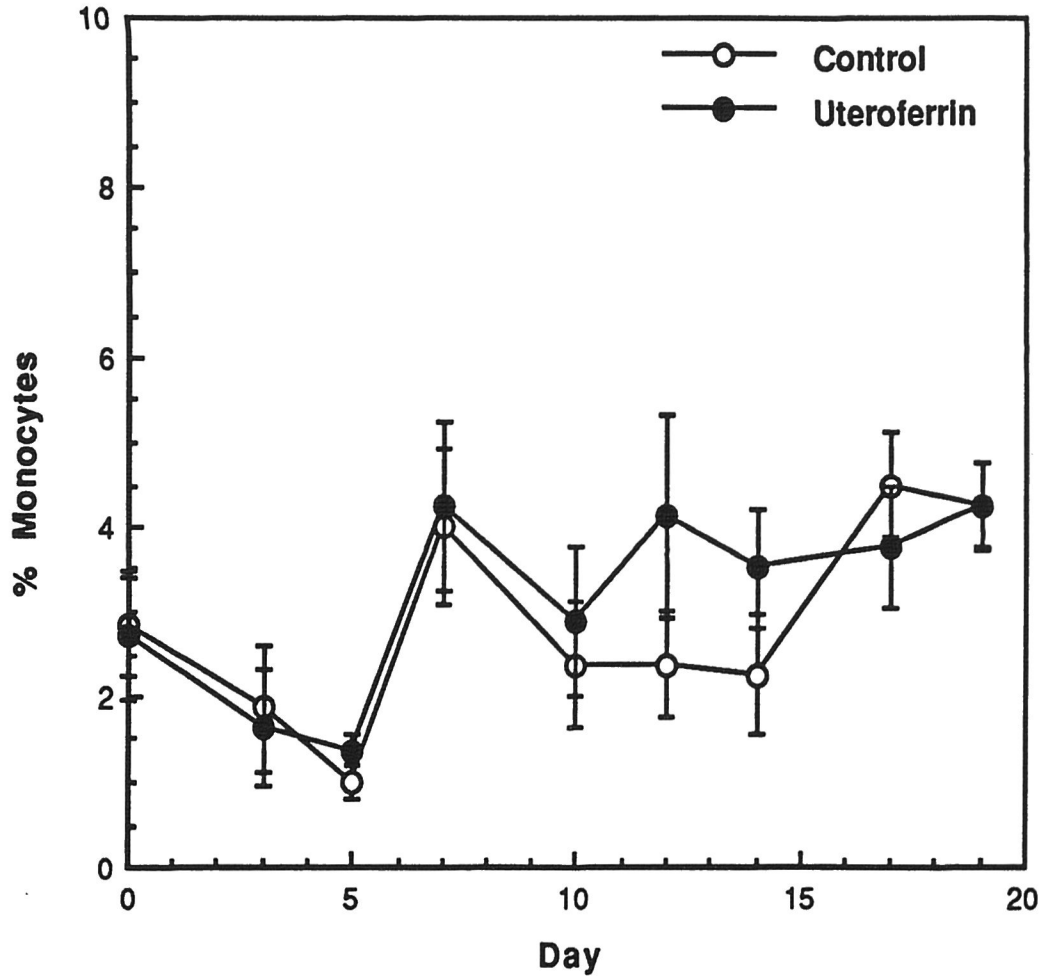

Figure 12. The effect of administration of uteroferrin on the percentage of monocytes in white blood cell differential counts from peripheral blood of young pigs. Pigs (initial weight 22.1 ± 1.5 kg) were assigned randomly to one of two treatment groups. Baseline counts were acheived via collection of peripheral blood on three consecutive days prior to the initiation of treatments (mean=d 0). Beginning on day 1 and throughout the course of the experiment, pigs received twice daily i.m. injections of uteroferrin (100 μg/kg in 0.9% NaCl) or an equivalent volume of 0.9% NaCl (control). Blood samples were taken three times/week and % monocytes determined. Points represent means±SEM from two separate experiments (n=8 total animals/treatment/time period). No significant effect of treatment was detected (P> 0.10)

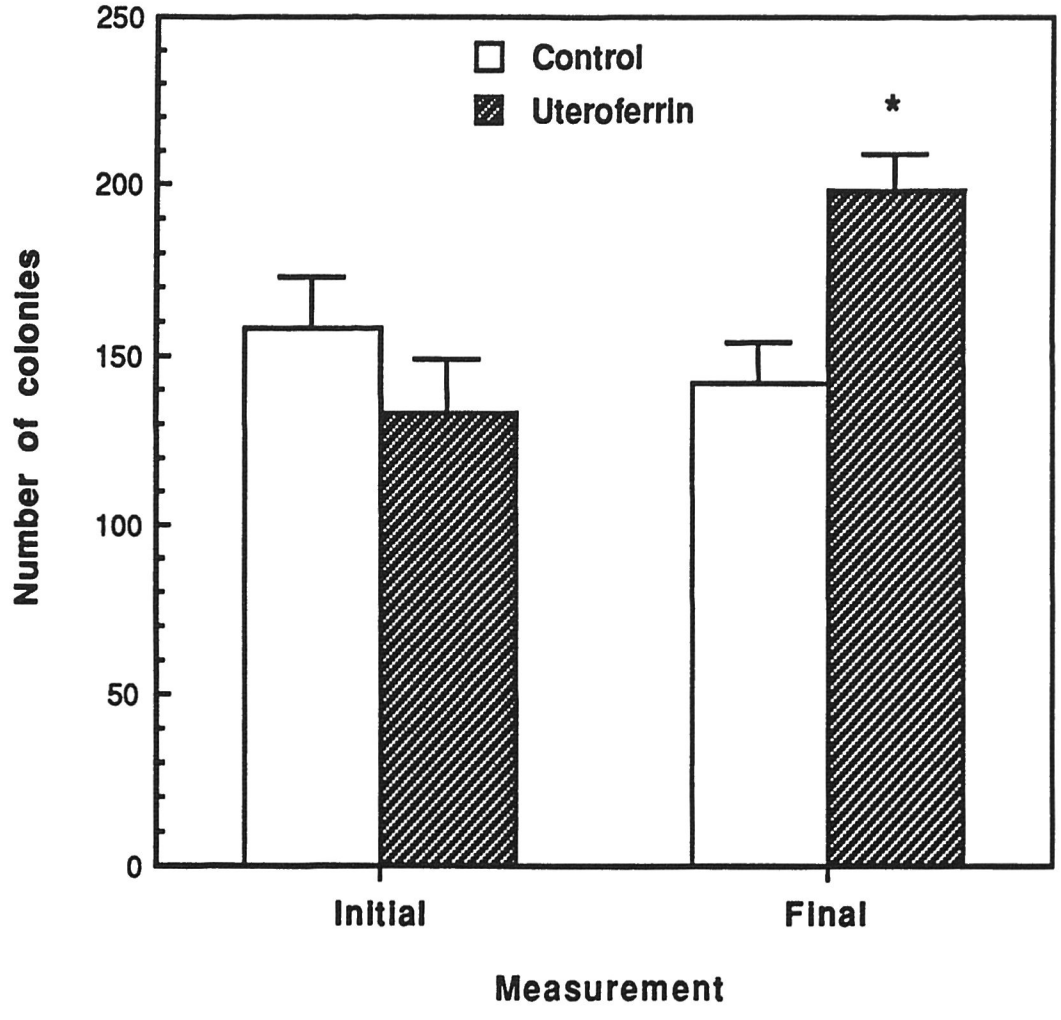

Figure 13. The effect of *in vivo* administration of uteroferrin on nonadherent peripheral blood cells assayed *in vitro* for BFU-E progenitor cells. Mature pigs (approximately 100 kg) were randomly assigned to one of two treatment groups. Following initial blood collection, pigs were injected i.m. with uteroferrin (70 μg/kg in 0.9% NaCl) or with an equivalent volume of 0.9% NaCl (Control). Peripheral blood was collected again 8 h post-injection. BFU-E assays were performed on nonadherent cells in 0.3% agar using 1 U rhEPO/dish as the stimulant. Values represent mean±SEM (n=2 animals /treatment). * Differs from initial ($P < 0.05$) and indicates a stimulatory effect of UF on BFU-E progenitor cells.

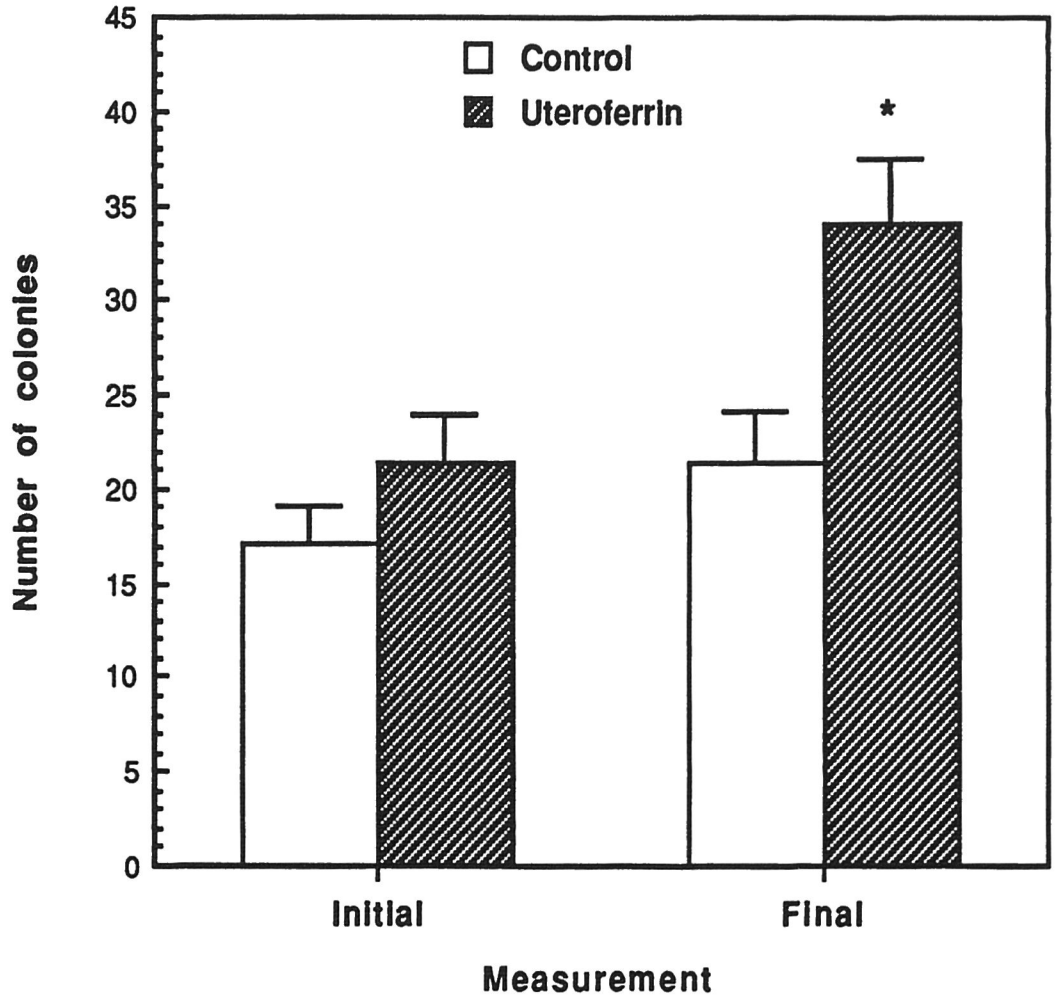

Figure 14. The effect of *in vivo* administration of uteroferrin on nonadherent peripheral blood cells assayed *in vitro* for CFU-GM progenitor cells. Mature pigs (approximately 100 kg) were randomly assigned to one of two treatment groups. Following initial blood collection, pigs were injected i.m. with uteroferrin (70 μg/kg in 0.9% NaCl) or with an equivalent volume of 0.9% NaCl (Control). Peripheral blood was collected again 8 h post-injection. CFU-GM assays were performed on nonadherent cells in 0.3% agar using 100 U rhGM-CSF/dish as the stimulant. Values represent mean±SEM (n=2 animals /treatment). * Differs from initial ($P < 0.05$) and indicates a stimulatory effect of UF on CFU-GM progenitor cells.

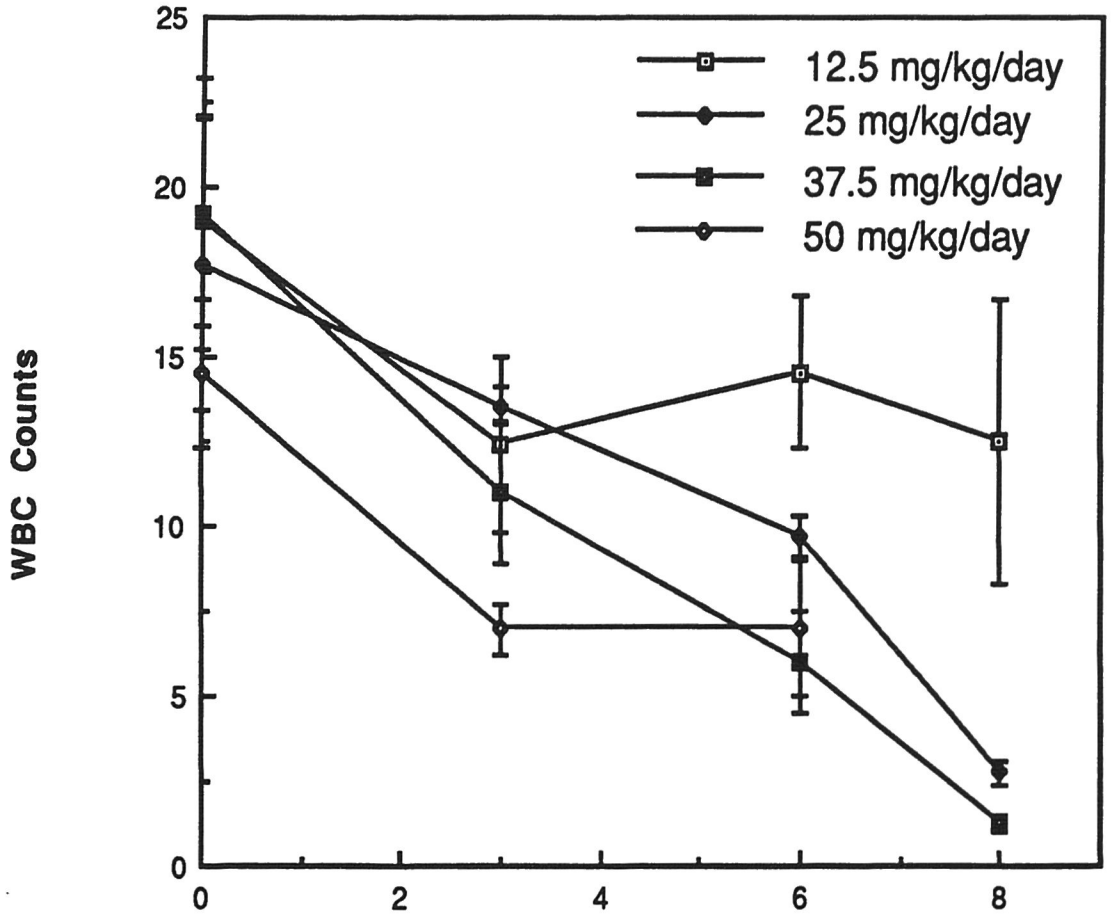

Figure 15. The effect of 5-FU on total WBC counts in young pigs. Gilts were assigned randomly to one of four treatment groups. Following establishment of a baseline value, pigs were infused i.v. with one of four doses of 5-FU (12.5, 25, 37.5, or 50 mg/kg/day on days 1 and 2). The results are discussed in the text.

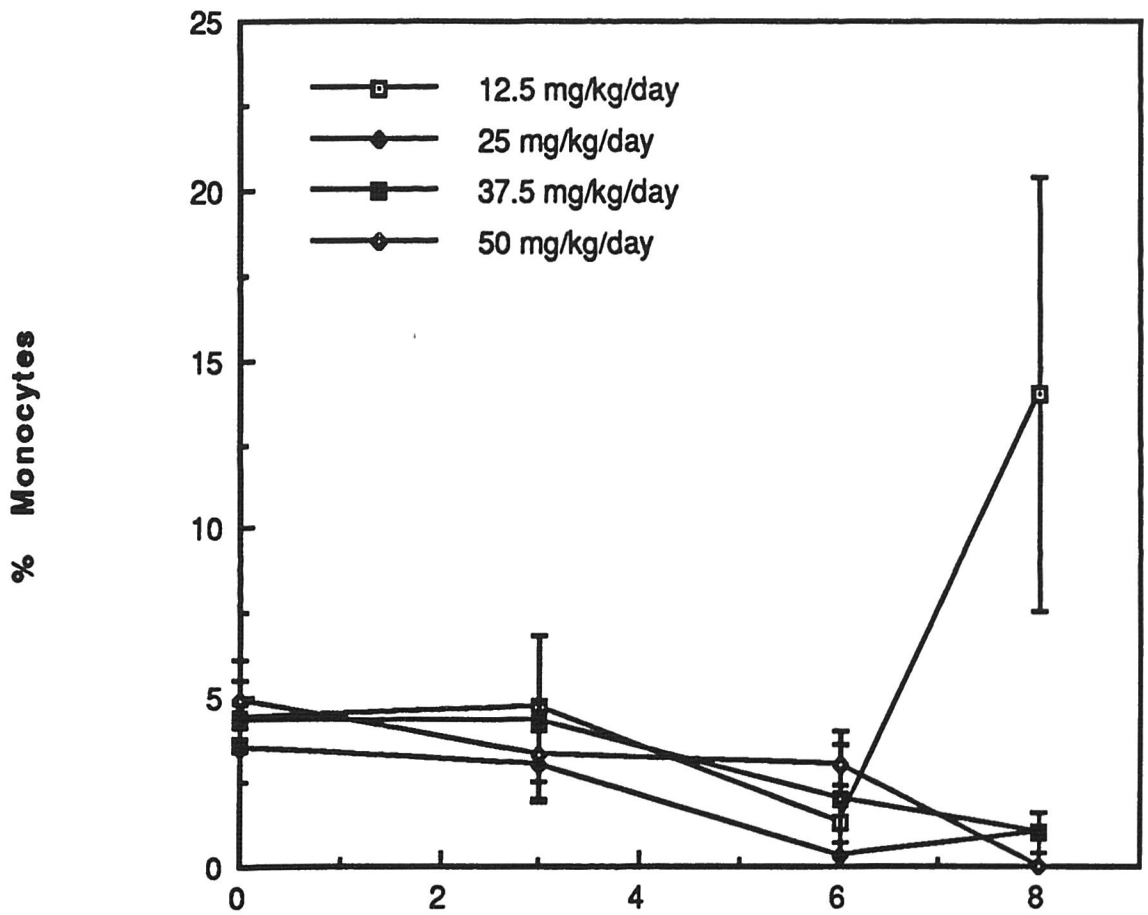

Figure 16. The effect of 5-FU on differential cell counts in young pigs. Gilts were assigned randomly to one of four treatment groups. Following establishment of a baseline value, pigs were infused i.v. with one of four doses of 5-FU (12.5, 25, 37.5, or 50 mg/kg/day on days 1 and 2). The results are discussed in the text.

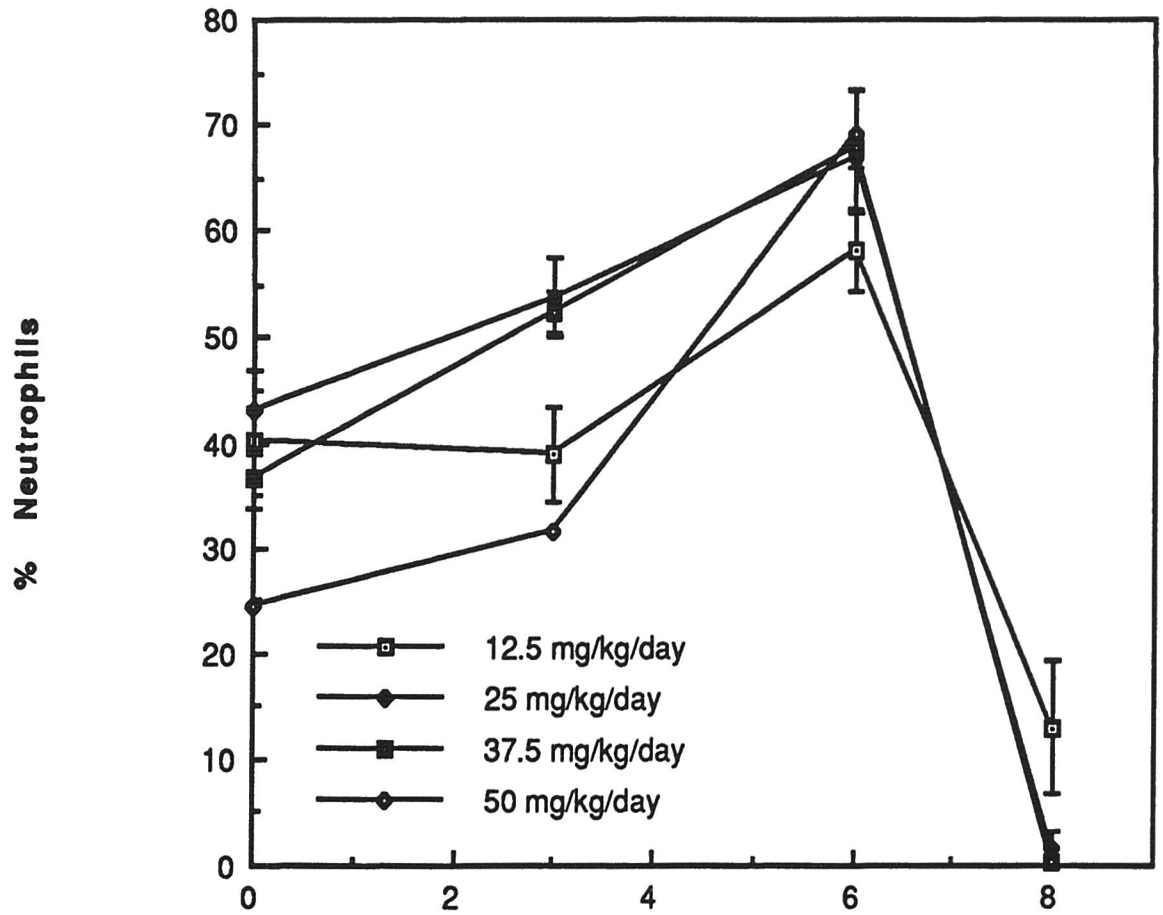

Figure 17. The effect of 5-FU on differential cell counts in young pigs. Gilts were assigned randomly to one of four treatment groups. Following establishment of a baseline value, pigs were infused i.v. with one of four doses of 5-FU (12.5, 25, 37.5, or 50 mg/kg/day on days 1 and 2). The results are discussed in the text.