Investigations on the blood composition of sows during the reproductive cycle

I. Blood changes during the oestrous cycle

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Contents: In the present series of experiments, 231 blood samples were collected from sows at different phases of the oestrous cycle and analysed for cellular, gaseous and biochemical blood parameters. Significant cyclical changes were noted for the total leucocyte count, for the pH-value, the HCO₃⁻ and total carbon dioxide concentration, the base excess, and the partial carbon dioxide pressure, the plasma protein and the blood glucose concentration. None of the blood enzymes tested nor the parameters related to the red blood cell count underwent significant cyclical changes.

Introduction

Blood has an important function as a mediator between the environment and the tissues and organs of the animal, as it supplies them with the nutrients, including oxygen and water, which are necessary for their optimal function. The constancy of the cellular, of the chemical and physical characteristics of blood is, consequently, a prerequisite of high productivity in farm animals. This homeostasis is, however, not absolute. Variations in environmental stress as well as those arising from endogenous demands for nutrients may cause, within relatively narrow limits, significant changes in blood composition. Reproductive functions constitute severe endogenous stress situations which involve the whole neuro-endocrine system, and which may therefore affect the amounts of cellular and chemical blood constituents. Despite the importance of blood for the maintenance and productivity of the individual animal, few attempts have been made to investigate blood changes other than endocrine during the oestrous or menstrual cycle (Southam and Gonzaga, 1965; Lethovirta, 1974; Mettler and Schirwani, 1974; Singh and Dutt, 1974; Vellar, 1974; Bain and England, 1975; Wrogemann, 1976), and only Goode, Warnick, and Wallace (1965) studied the level of alkaline phosphatase in the pig during the oestrous cycle.
It was therefore desirable to measure a fairly large number of blood parameters at various stages of the oestrous cycle of adult sows in order to estimate the occurrence and magnitude of variations in the cellular and chemical components of the blood, which might be indicative of hormonal changes during the different phases of the oestrous cycle.

Materials and Methods

The present studies were carried out in the humid-tropical climate of south-western Nigeria, where the mean annual temperature comes to 26.6 °C and the relative humidity reaches an average of 71%. The slight, though significant, changes in the climatic variables were eliminated for the purpose of this study by replicating the blood analysis during each of the four quarters of the year.

The pigs were of European origin — Large White x Landrace crossbred sows, in which the Large White component dominated — which had for several generations been maintained in the breeding herd of the Teaching and Research Farm of the University of Ibadan. They were fed ad libitum with a ration considered suitable for breeding pigs in a tropical climate (Babatunde, 1972). The sows were checked for heat daily with a vasectomized boar over at least one whole cycle before the bleeding schedule was begun. During the oestrous cycle blood samples (10–15 ml) were collected into heparinized test tubes on days 2, 11, 14, 19 and 21, day 1 being the first day of standing heat. Samples were stored on ice, in the refrigerator (+4 °C) and/or in the freezer (−15 °C) until they were analysed in duplicate by standard methods (Tewes, 1977) for packed cell volume (Ht), haemoglobin (Hb), number, volume (MCV) and haemoglobin content (MCH) of erythrocytes, number of leucocytes and differential cell count, pH-value, partial pressure of oxygen (pO₂), plasma hydrogen carbonate (HCO₃⁻), total carbon dioxide, base excess, total plasma protein, blood glucose, inorganic phosphorus, and the enzymes lactic dehydrogenase (LDH) and alkaline phosphatase (AP). The number of samples analysed were 56, 64, 46, 36 and 29 for days 2, 11, 14, 19 and 21, respectively; thus the study involved a total of 231 blood samples all of which were analysed for 23 different blood values.

The means of duplicate observations were subjected to a least squares analysis testing for main effects, interactions with season as well as for linear, quadratic, cubic and quartic influences of the stage of the oestrous cycle on the blood value under consideration. As there was no interaction between season and stage of the cycle, with the exception of the haematocrit and the percentage of monocytes in the differential leucocyte count, the effect of season will not be discussed in the present paper. The values given below will be the means and standard errors of the means unless stated otherwise.

Results and Discussion

1. The red blood cell and associated phenomena

None of the parameters associated with the erythrocytes was subject to cyclical variation. The haematocrit value averaged 42.7 ± 0.3 %, a value slightly higher than in investigations from temperate climates. On the other hand, the red blood cell count (5.3 ± 0.06 x 10⁶/mm³) is slightly lower than reported elsewhere, and consequently the mean corpuscular volume (81.9 ± 0.9 μm³) is increased by about 30 percent. Without concurrent determinations of the plasma or blood volume, it is difficult to decide whether these deviations have to be attributed to the tropical climate. The slightly increased haemoglobin content (14.5 ± 0.14g/100 ml) and the greatly elevated mean corpuscular haemoglobin (27.9 ± 0.36 pg/rbc) suggest a certain degree of haemoconcentration that might be due to a climatic stress-induced change in adrenocortical physiology, and therefore a change in the electrolyte and water equilibrium. Adrenocortical hypertrophy in heat-stressed gilts has been observed recently (Steinbach, unpublished).
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2. The total number and differential count of leucocytes

The total leucocyte count averaged $11.6 \pm 0.23 \times 10^3 / \text{mm}^3$, which is similar to values reported elsewhere. There was a significant ($F = 2.38, P < 0.05$) and quadratic effect of the stage of the oestrous cycle on the number of leucocytes (Fig. 1). The high leucocyte values on day 14 and 19 of the cycle indicate a relationship between luteolysis and this parameter. Wrogemann (1976) reported observations slightly different from the present results made on miniature pigs which indicate an increase in leucocyte numbers during the prooestrous and oestrous periods. Significant cyclical variations in the other investigated leucocytic parameters were not demonstrated. The total number of eosinophilic granulocytes ($373 \pm 21 / \text{mm}^3$) was less, their proportion in the differential leucocyte count ($3.9 \pm 0.21 \%$) was, however, greater than other published values. Similarly, the percentage of the lymphocytes was much higher ($69.0 \pm 0.8 \%$), at the expense of the neutrophilic granulocytes ($24.6 \pm 0.8 \%$). The significance of this shift in favour of lymphocytes and eosinophilic granulocytes is not clear. The mean percentages of monocytes ($2.0 \pm 0.13 \%$) and basophilic leucocytes ($0.38 \pm 0.05 \%$) were close to values obtained elsewhere.

3. The acid-base equilibrium

The acid-base equilibrium is maintained within rather narrow limits through renal and respiratory function. Changes in the water and electrolyte metabolism or in respiratory frequency will affect the acid-base balance as well. The pH-value was $7.32 \pm 0.01$ on average, with significant ($F = 2.94, P < 0.05$) cubic variations throughout the oestrous cycle, with the lowest level during the prooestrous period (Fig. 2) and was thus either a consequence of the luteolytic processes mentioned above or preceding and possibly inducing the commonly observed fall in vaginal pH during oestrus. The concentration of hydrogen carbonate ($27.8 \pm 0.29 \text{mM/l}$) and total carbon dioxide ($29.7 \pm 0.30 \text{mM/l}$) as well as the base excess ($1.1 \pm 0.33 \text{mEq./l}$) followed roughly the same pattern during the oestrous cycle as the blood pH, with minimum values on day 19, during the prooestrous period (Fig. 3, 4 and 5). These cyclical variations were highly significant ($P < 0.01$). By contrast, the highest partial pressure of carbon dioxide was observed on day 14 (Fig. 6), following which it declined steadily and reached a low value during or just before oestrus (day 21). These results indicate a respiratory depletion of carbon dioxide during the later part of the oestrous cycle, since Okhawere and Steinbach (unpublished) observed a development of the respiratory rate of heat-stressed gilts during the oestrous cycle (Fig. 7) which ran exactly opposite to the concentration of hydrogen carbonate, of total carbon dioxide and of base excess (Fig. 1, 3, 4 and 5). Also, in the present series of investigations highly signifi-

![Fig. 1: Cyclical changes in the blood leucocyte count](image-url)
Fig. 2: Cyclic changes in the pH-value or blood

Fig. 3: Cyclic changes in the plasma bicarbonate concentration

Significant negative correlations were observed between the respiratory rate on the one hand and pH-value, bicarbonate or carbon dioxide concentration or base excess on the other, and the magnitude of the negative regression coefficients which apparently increased with increasing respiratory rate (Tewes, 1977). The physiological reasons of this increased respiratory rate and the subsequent acidosis, resulting apparently from an inadequate buffering capacity of the CO₂-depleted blood during the luteolytic and early follicular phase of the oestrous cycle, is not entirely clear. The mean partial oxygen pressure (pO₂) amounted to $38.0 \pm 0.5$ mm Hg, a value comparable to those reported from elsewhere. But in spite of the changing pCO₂, there were no variations during the oestrous cycle.
4. Chemical blood components: plasma protein, blood glucose and inorganic phosphorus

With $7.20 \pm 0.04$ g total protein / 100 ml plasma, the average value agrees closely with other published reports. During the oestrous cycle, there were significant variations ($F = 3.02, P < 0.05$), and the response curve was of a quadratic nature (Fig. 8) with a high value at ovulation time (day 2) and a minimum at the end of the luteal phase of the oestrous cycle (day 14). This curve is very similar to that of the water content in the endometrium (Steinbach, 1972) or to that of epithelial changes in the vagina (Steinbach, unpublished). These similarities would indicate a dominating influence of oestrogens on the level of total proteins in the plasma. Indeed, Raeside (1963) and Lunaas (1962) reported peak urinary oestrone excretion in the sow on days 20 and 1,
respectively, with a gradual increase setting in before day 15. This observation as well as the finding that there is a latent period of 24–48 h between the administration and the urinary excretion of oestrogens (Terqui, Rombauts and Fèvre, 1968) seem to suggest that the changes in plasma proteins reflect the oestrogen status of the animal, although it is not known by which endocrine mechanism these two are linked together.

The average blood glucose level of 83.3 ± 1.0 mg/100 ml was within the normal range. There, too, is evidence of oestrogenic stimulation: the level of glucose remained low during the luteal phase, increased rapidly between days 14 and 19, i.e. following luteolysis, decreasing thereafter, during the heat period (Fig. 9). These changes were highly significant ($F = 6.64, P < 0.01$). This relationship between gonadal status and blood glucose level may be mediated by the adrenal cortex: Gemzell (1953) suggested that oestrogens stimulate the adrenal cortex by inducing the release of ACTH from the adenohypophysis, and Steinbach (unpublished) observed recently an increase in adrenocortical width on days 19 and 1, i.e. the prooestrous and oestrous periods, during the hottest time of the year in a tropical environment. In view of the findings

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**Fig. 6:** Cyclical changes in the partial carbon dioxide pressure in the blood

**Fig. 7:** Changes in the respiratory rate of heat-stressed gilts (hot season, afternoons) during the oestrous cycle (after Okhawere & Steinbach, unpubl.)
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of Bianca and Findlay (1962), who observed a positive relationship between respiratory and blood glucose levels in calves, the earlier-mentioned observations of Okhawere and Steinbach (Fig. 7) are noteworthy: the blood glucose curve in the present paper (Fig. 9) parallels closely the one depicting the variations in respiratory frequency (Fig. 7). However, these two consequences of high oestrogen titres are not likely to be directly related. As indicated above, oestrogens affect the carbohydrate mobilization via the hypothalamic-hypophyseal-adrenal axis, but induce an increased respiratory rate by way of the increased activities which are typical of prooestrous and oestrous sows (Altmann, 1941).

The inorganic phosphorus concentration averaged $7.35 \pm 0.05 \, \text{mg/100 ml}$, a level which is higher than those in other reports. This is possibly a consequence of the constant thermal stress, since Schmidt et al. (1970) recorded an increase in the concen-
tation of phosphorus in heat-stressed stress-susceptible pigs, and since Hartig (1974) observed high values in growing pigs during the hot season in Nigeria under similar experimental conditions. There were no significant cyclical variations in the phosphorus content.

5. Blood enzymes: Lactate dehydrogenase and alkaline phosphatase
The average activity of lactate dehydrogenase (LDH) was 283 ± 4 mU/ml, and thus slightly above the normal range of this parameter. Many studies have shown that a number of different stress situations, both acute and chronic, may increase plasma LDH levels due to an increased permeability of the cell walls. For instance, Haase (1971) reported increased LDH values for heat-stressed pigs, and Tewes (1977), in the present series of experiments, found a positive correlation between the average temperature during the week prior to bleeding and the LDH activity. However, the variations of LDH during the oestrous cycle were small and not significant.

Similarly, the level of alkaline phosphatase failed to demonstrate cyclical variations. This result is in disagreement with the finding of Goode et al. (1965), who reported an increase in the activity of this enzyme during oestrus.

References

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