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Immunological quantitation of IgG and IgM in milk and serum of the goat at different stages of the reproductive cycle

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ABSTRACT

IgG and IgM levels in colostrum, milk and serum were quantitated by radial immunodiffusion. Concentration of milk IgG was found to be 65 mg/ml at the colostrum period. It further decreased to 3 mg/ml after Day 15 of lactation. However, the concentration of serum IgG was constant throughout the reproductive cycle (8-11 mg/ml). Concentrations of IgM in Serum and milk range between 0.5-1.5 mg/ml and 0.2-04 mg/ml respectively, with slight higher values in serum. Hence, IgG was rather used for offspring immunity than for the defense of the mammary gland.

Keywords: IgG, IgM, immunoglobulins, goat,single radial immunodiffusion

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INTRODUCTION

The mammary gland not only provides a vital nutrient source to the offsporings, but also serves as a necessary source of immunoglobulins. Immunoglobulins in the mammary gland and secretion can be either derived from the blood circulation or produced locally by plasma cells underlying the epithelium. In ruminants, there is no transplacental shift of immunoglobulins to the fetuses but they are passively transferred immediately postpartum [3, 8].

Many authors have determined the concentrations of different classes of immunogloubulins in the colostrum and milk of the cow [6, 7, 13, 14,]. and human [4].

On the other hand, to date, no report is available for immunoglobulins in the milk and serum of the goat. Hence the present study was carried out to determine the concentration of 1gG and 1gM in the milk and serum of the goat immunochemically to establish normal values. This may be useful in comparison with pathological conditions. The data on the immunoglobulin concentrations in the colostrum, milk and serum was correlated with the relative frequency of plasma cells as a prerequisite to understanding the mechanism of humoral immunity.

MATERIALS AND METHODS

Animals
Milk (refer to colostrum and milk) and blood were collected from 4 non-pregnant (resting period) 6 pregnant (mid- and late pregnancy) and 15 lactating (colostrum period and 15, 30, 60 and 90 days after parturition) Saanen goats. All animals were clinically normal at the time of sampling.

Quantitative immunoglobulin determination

Serum was prepared from collected blood and stored at -20Cº. Samples of milk and colostrum were clarified by high speed centrifugation (38,000 g for 30 min) and stored the same way as serum samples. The single radial immunodiffusion method of Mancini et al. [11] was adopted for measuring immunoglobulin concentration. Briefly, the antisera against IgG or IgM, diluted at 1:100, were mixed with an equal volume of 3% purified agar making a final 1.5% agar solution. The agar was then poured in dishes so as to make one millimeter thick layer, Thereafter, the standard antigens at serial dilutions, milk or serum were applied in the different wells made in the agar. Then the precipitated ring diameter was measured after including for four days at room temperature (RT). Data are expressed in units of concentration (mg/ml) for both serum and milk.

Antibodies and antigens

Anti-goat IgG (Cappel) and anti-goat IgM (Bethyl Lab.) were used as antibodies. Goat IgG and IgM antigens (chemicon int CA. USA) were obtained for blotting of the standard graph (Fig.1A,B). The mean and SD were calculated and comparison between various stages was made using students t-test.

RESULTS

Immunoglobulins

The concentration of IgG in all samples was found more than that of IgM (Fig.1A, B). The concentrations of serum IgG were rather constant throughout the different stages of the reproductive cycle ranging from 8-11 mg/ml [Fig.1A and Fig2 (3,4)]. In contrast, serum IgM concentrations (range between 0.5-1.5 mg/ml) were variable with some fluctuation at the different stages of the reproductive cycle [Fig.1B and Fig.2 (6)].

Concentration of milk IgG was high at late pregnancy and further increased at the colostrum period (65 mg/ml) but decreased significantly after day 15 of lactation (3mg/ml) [Fig. 1A and Fig.2 (1, 2)]. Then it slightly increased at the resting period. Milk IgM was constant throughout the reproductive cycle (0.2 mg/ml), although a slight increase (0.4 mg/ml) was evident at the colostrum period [Fig.1B and Fig.2 (5)].

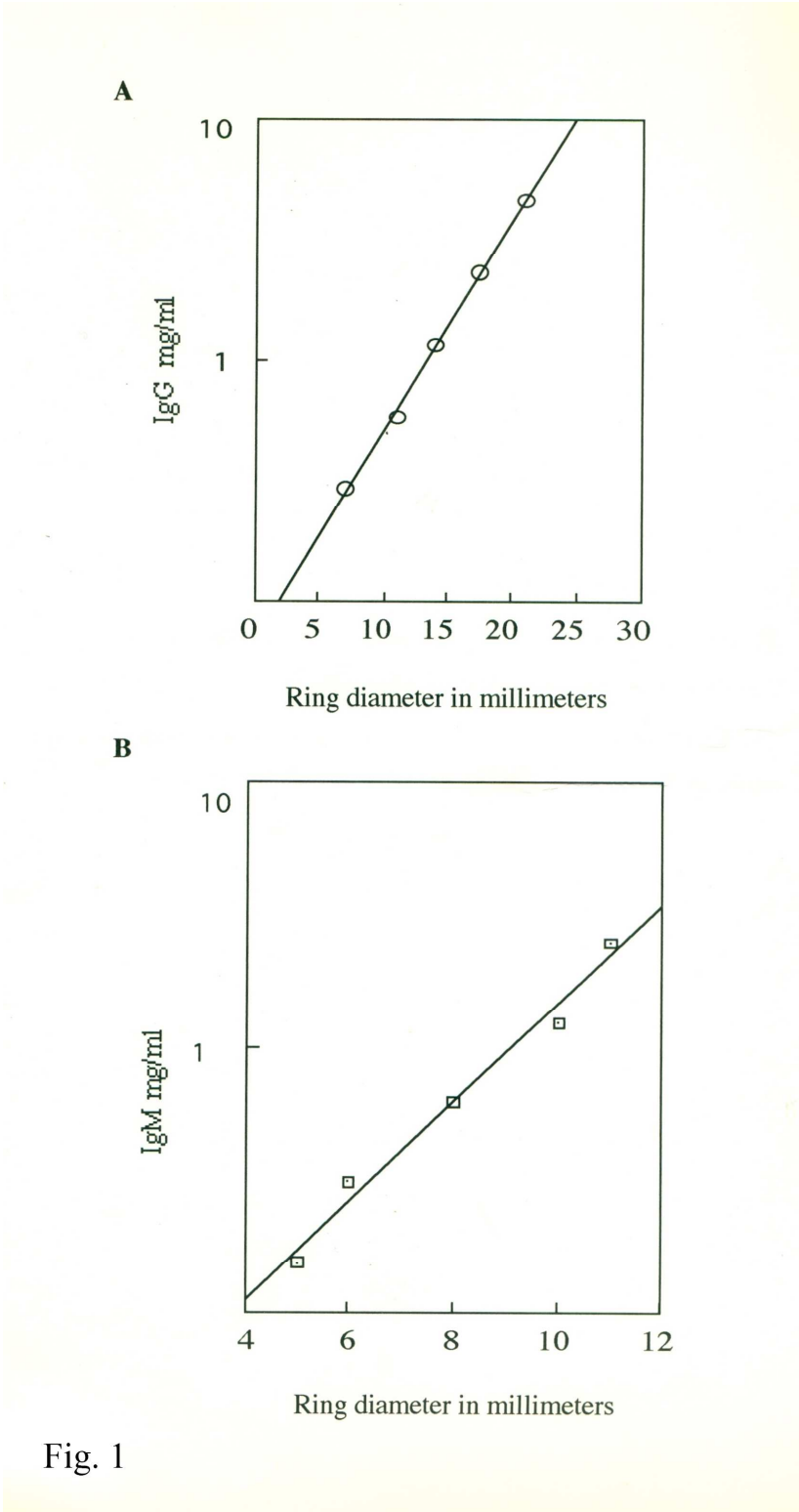

Fig. 1: (A) A representative graph comparing IgG concentration (logarithmic scales) vs. the precipitate ring diameter. (B) A representative graph comparing IgM concentration (logarithmic scales) vs. the precipitate ring diameter.

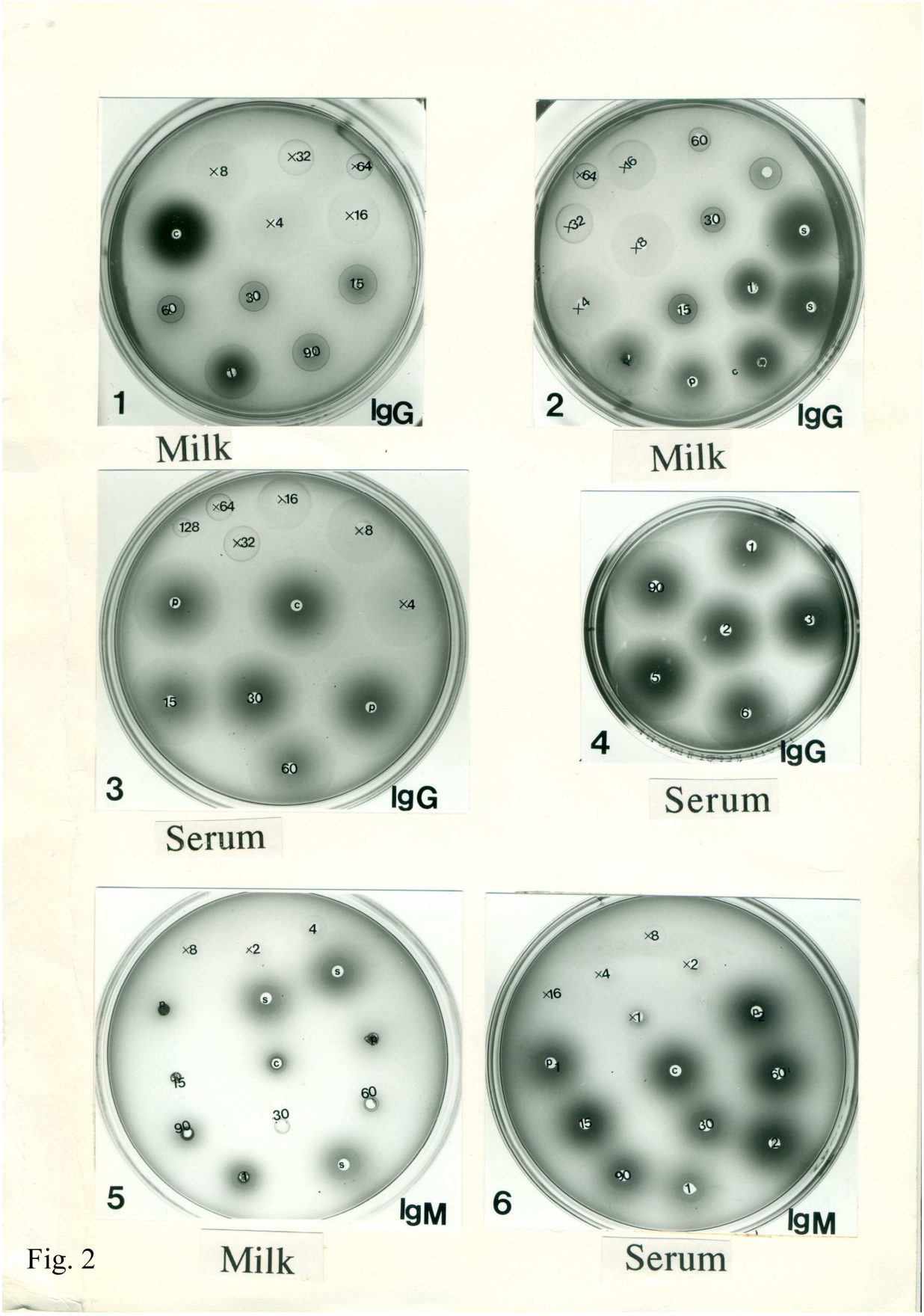

Fig. 2: Example of antigens measurement. The wells contain dilution of the antigens, the precipitation zones are stained with Coomassie blue.

DISCUSSION

1gA is the major immunoglobulin in the colostrum of humans and rodents [1, 2, 5, 4, 8, 19, 20]. In the present study the measurement of the 1gA concentration could not be done due to the difficulty of obtaining pure 1gA for plotting the standard graph. However, an immunodiffusion experiment showed that it is a minor immunoglobulin in the goat colostrum or serum (Ismail, unpublished data). This holds true for sheep and cow in which only low amounts of 1gA are present in colostrum [17].

IgG was found in the present study to be the predominant immunoglobulin in milk and serum. Its concentration increased at late pregnancy peaked at the colostrum period and sharply decreased during lactation. These findings confirm the result obtained in other ruminants and tow where 1gG is the main immunoglobulin in colostrum [3, 8, 13, 17]. The paucity of 1gG containing plasma cells in the goat mammary gland (Ismail, unpublished data) in comparison to the high concentration of 1gG in the milk may lead to the suggestion that 1gG in the goat mammary gland is largely serum derived, which is consistent with the findings in the cow and sheep [9, 12.

In this study the goat colostrum contained 65 mg/ml of 1gG and 0.4 mg/ml of 1gM. Similar ranges of 1gG in the colostrum were also given for the sheep and cow whereas the value of IgM in the goat colostrum was a little lower than in other ruminants [10, 18,]. The levels of both 1gG and 1gM decreased after lactation day 15. The rapid fall in immunoglobulin contents of the colostrum is also recognizable in other ruminants for all immunoglobulin [15, 16].

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