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Early Pregnancy Diagnosis in Water Buffalo by Early Pregnancy Factor Measurement Using Rosette Inhibition Test

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Abstract: The diagnosis of pregnancy is essential for profitable animal husbandry especially in the productive animal species. Early pregnancy factor (EPF) was detected in the serum of all mammals tested within 24 to 48 h of fertilization and disappeared within 24 to 48 h after death or removal of embryo. EPF activity may be useful as an indirect method of pregnancy diagnosis. Blood samples from 50 artificially inseminated (AI) and 10 non-inseminated (control) Iranian water buffalo (*Bubalusbubalis*) were collected and allowed to clot. Serum was collected and stored at -20°C for assay. Test of pregnancy (rectal palpation) was done after 50th day. Rosette inhibition test (RIT) was performed to evaluate the EPF activity. RIT values were significantly higher ($P < 0.05$) in pregnant (8.00 ± 0.13) than in non-pregnant (2.71 ± 0.21) cows. In conclusion, the RIT score for EPF detection can be used as a tool for early detection of pregnancy or embryonic death in buffalos.

Key word: Early Pregnancy Factor · Rosette Inhibition Test · Water Buffalo

INTRODUCTION

The diagnosis of pregnancy is essential for profitable animal husbandry especially in the productive animal species. For an economically dairy farm, animals must calve every year and to maintain this, identifying pregnant animals at an early date seems imperative. In the current systems of planned breeding, diagnosis of early pregnancy would help to evaluate the therapies at an early dateand devise alternative manipulations. It therefore, appears that early diagnosis of pregnancy is essential in animal management for economic reasons.There is a need to check animals for pregnancy at an early date as it has been shown that earlier the pregnancy diagnosis performed, the more profitable is the return for dairy cows and buffaloes [1].

Early pregnancy factor protein molecule was first identified in pregnant mice [2] and in sheep and cattle [3] by using the rosette inhibition bioassay. With this assay, EPF was detected in the serum of all mammals tested within 24 to 48 h of fertilization and disappeared within 24 to 48 h after death or removal of embryo [2]. The developing embryo bears antigens foreign to the mother; hence immune rejection of the early embryo may occur. An immunosuppressive early pregnancy factor (EPF) appears as early as 6 to 48 h of mating which functions to suppress the maternal immune response thereby allowing for pregnancy to proceed [4]. In cattle significant differences in rosette inhibition titer were observedbetween pregnant and non pregnant cows on day 13-16 and 25 post breeding [5],suggesting that measurement of EPF activity may be useful as an indirect method of pregnancy diagnosis [1]. The aim of present study was to detect the pregnancy in early stage in water buffalo.

MATERIALS AND METHODS

Animals and Serum Collection: Blood samples from 50 artificially inseminated (AI) and 10 non-inseminated (control) Iranian water buffalo (*Bubalusbubalis*) were collected from the jugular vein by venoject in two sets:

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3rd day and 7th day; and allowed to clot. Serum was collected by centrifugation at 1000 g for 30 min, inactivated by heating at 56°C for 30 min and stored at -20°C for assay. Test of pregnancy (rectal palpation) was done after 50th day.

Isolation of Lymphocytes: The lymphocytes from male water buffalo (lymphocyte donor) were separated by Ficol (Pharmacia, Sweden) density gradient centrifugation, according to manufacturer instruction. Lymphocyte cells were washed with PBS by centrifugation at 450 g for 5 min. The final suspension of the lymphocytes was adjusted to 1.5×10^7 cells/ml with PBS.

Sheep Red Blood Cells (SRBC): Peripheral blood of male sheep was collected in heparin as anticoagulant and centrifuged at 1600 × g for 10 min. The erythrocytes were washed five times with PBS. The final suspension of the SRBC was 1% in PBS and stored at 4°C till further use [6].

Estimation of the Rosette Formation Ratio: The rosette inhibition test (RIT) was performed following the protocol previously described [6-8] with minor modifications. Test serum samples were serially diluted to a final volume of 200 µL with PBS. Equal volume of lymphocyte suspension (1.5×10^7 cells/ml) was added and incubated at 37.5 °C for 60 min.

Serum samples of each animal (100 µl) were mixed with lymphocytes suspensions (500 µl) and incubated at 3°C for 60 min. The reaction solutions were divided into twelve equivalents (50 µl each sample), then 100 µl serially diluted ($1 : 2^1 \times 10^3$, $1 : 2^2 \times 10^3$, to $1 : 2^{12} \times 10^3$) rabbit anti lymphocyte serum (ALS) were added and incubated at 37°C for 60 min, washed 3 times with Hanks', respectively.

The rosette formation was initiated by addition of 200 µl of 1% SRBC and further incubated at 37.5°C for 5 min. Centrifuged at 400 g for 5 min. Samples were incubated for 15 min at room temperature and re-suspended gently. Then, 0.03 ml of 10% glutaraldehyde was added to fix the lymphocytes and erythrocytes. The rosette formations with tight adherence of erythrocytes to lymphocytes were recorded. The RI titer was expressed as the negative base two logarithm of the highest dilution of serum for which the number of rosettes was 75% or less that seen in the control tubes with no serum [8].

Statistical Analysis: Rosette-inhibition titers were analyzed using the SPSS Statistics 16.0 ANOVA and tukey post hock test. The data presented as mean ± SEM (P<0.05).

RESULTS

RIT values ranged from 7 to 9 in the serum of pregnant cows, while in controls it ranged from 2 to 4 in samples collected at 3rd day (Fig. 1). The mean values were significantly higher ($P < 0.05$) in pregnant (8.00 ± 0.13) than in non-pregnant (2.71 ± 0.21) cows. In 7th day samples, the RIT values in serum of pregnant cows ranged from 7 to 9, while in controls it was recorded 2-4 (Fig 2). The mean RI titer values were significantly higher ($P < 0.05$) in pregnant (7.68 ± 0.15) than non-pregnant (2.65 ± 0.14) cows. The RIT value did not increased significantly ($P>0.05$) with days after pregnancy in pregnant cows (8.00 ± 0.13 vs. 7.68 ± 0.15).The RIT values

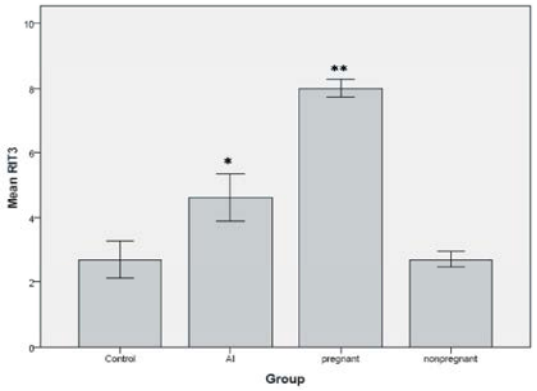

Fig. 1: RIT values in the serum of cows at 3rd day after AI. RIT: Rosette inhibition test. AI: Artificial insemination. *: Significant differences in comparison to control (P<0.05). **: Significant differences in comparison to control (P<0.01).

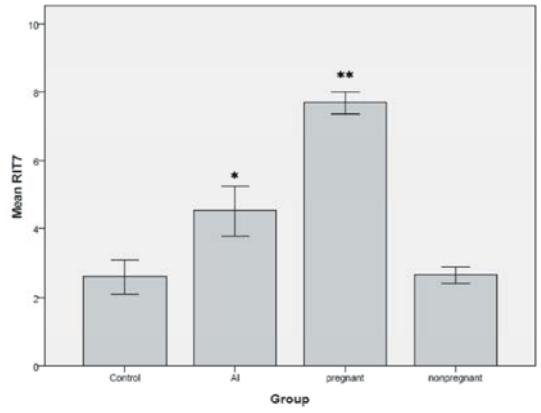

Fig. 2: RIT values in the serum of cows at 7th day after AI. RIT: Rosette inhibition test. AI: Artificial insemination. *: Significant differences in comparison to control (P<0.05). **: Significant differences in comparison to control (P<0.01).

in AI cows were different from pregnant cows, because the pregnancy rate of AI in water buffalo is low, especially in summer (the season that samples were taken).

DISCUSSION

In this study, we observed that the RIT score of pregnant buffalos was significantly higher than control and inseminated but not pregnant buffalos. The mean RIT was more than twice that of all non-pregnant buffalos. RIT score did not increase within few days after insemination. The titer of 7th day samples was not higher than that from 3rd day pregnant cows, which is in contrast with results of the experiments on Shetland pony [9] and cows [6]. The presence of EPF has been repeatedly confirmed as indispensable factor to confirm successful pregnancy [6,10,11]. EPF activity has been detected by RIT within 1 week after mating and/or ovulation in mouse [12], equines [9], porcine [8] and cow [6]. It has been shown that the mean RIT value declined rapidly after abortion within 7 days, to the level of EPF that is present in non-pregnant women [11]. Similar trend was reported for embryo donor mares [13]. RIT has been used to confirm pregnancy and detection of early embryonic death [3].

The RIT test was based on the ability of serum containing EPF to inhibit the formation of spontaneous rosettes between lymphocytes and SRBC. This means that the lymphocytes are spontaneously form rosettes, a flower-like arrangement in which a lymphocyte has several red blood cells attached to it. Lymphocytes from pregnant animals form fewer rosettes than those from non-pregnant animals. Therefore, with the aid of EPF diagnostics, a pregnancy can be detected at a very early stage. Based on our results, RIT score above 7 for EPF assay can be used for confirmation of pregnancy and less than for 3 for non-pregnant buffalos. Hence, use of RIT for EPF detection is convenient, practical and speedy technique for early detection of pregnancy as compared to skill-based rectal palpation of viscera.

In conclusion, the RIT score for EPF detection can be used as a tool for early detection pregnancy or early embryonic death in combination with other techniques in water buffalos.

REFERENCES

1. Purohit, G., 2010. Methods of Pregnancy Diagnosis In Domestic Animals: The Current Status.Webmed Central REPRODUCTION, 1(12): WMC001305.
2. Morton, H., B.E. Rofle and A.C. Cavanagh, 1987. Ovum factor and early pregnancy factor. Current Topics in Developmental Biology, 23: 73-92.
3. Nancarrow, C.D., A.L.C. Wallace and A.S. Greswal, 1981. The early pregnancy factor of sheep and cattle. Journal of Reproduction & Fertility, 30: 191-199.
4. Shaw F.D. and H. Morton, 1980. The immunologicalapproach to pregnancy diagnosis : a review. Veterinary Record, 106: 268-270.
5. Sakonju, S., S. Enomoto. S. Kaminura and K. Amana, 1993. Monitoring bovine embryo viability with 698 earlypregnancy factor. The Journal of Veterinary Medical Science, 55: 271-274.
6. Ghaffari, L.V., L.R. Ghaffari, N. Pirany and A.M. Moghadaszadeh, 2008. Measurement of EPF for detection of cow pregnancy using rosette inhibition test. Theriogenology, 70: 105-107.
7. Ohnoma, K., K. Ito, Y.I. Miyake, J. Takahashi and Y. Yasuda, 1996. Detection of early pregnancy factor (EPF) in mare sera. Journal of Reproduction and Development, 42: 23-8.
8. Quan, K., X. Zhao. Ch. Zhang. Q. Xu. H. Wei. J. Hu and Y. Zhang, 2011. A novel approach for very early pregnancy diagnosis in swine by anti-early pregnancy factor (EPF) antiserum blocking enzyme-linked immunosorbent assay (ELISA). African Journal of Biotechnology, 10(47): 9710-9716.
9. Ohnuma, K., M. Yokoo, K. Ito, J. Takahashi, Y. Nambo and T.I. Miyake, 2000. Study of early pregnancy factor (EPF) in equine (Equuscaballus). American Journal of Reproductive Immunology, 43: 174-9.
10. Cheng, S.J., A.Y. Ma, C.X. Qiao and Z.Q. Zheng, 2000. Early pregnancy factor activity in serum of patients with surgical abortion. American Journal of Reproductive Immunology, 44: 211-3.
11. Cheng, S.J. and Z.Q. Zheng, 2004. Early pregnancy factor in cervical mucus of pregnant women. American Journal of Reproductive Immunology, l51: 102-5.
12. Athanasas-Platsis, S., H. Morton, G.F. Dunglison and P.L. Kaye, 1991. Antibodies to early pregnancy factor retard embryonic development in mice in vivo. Journal of Reproduction Fertility, 92: 443-51.
13. Sakonju, I., S. Enomoto. S. Kamimura andK. Hamana, 1993. Monitoringbovine embryo viability with early pregnancy factor. The Journal of Veterinary Medical Science, 55: 271-4.