CUTANEOUS BASOPHIL HYPERSENSITIVITY REACTION TO PHYTOHEMAGGLUTININ IN HAIFERS AND COWS WITH DIFFERENT REPRODUCTIVE RESULTS

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We investigated the general immune reactivity, as estimated by the cutaneous basophile hypersensitivity reaction (CBHR) to phytohemagglutinin (PHA) in cows with different reproductive results. The hypersensitivity test was performed on the day of artificial insemination and animals were divided into groups according to the total number of inseminations, number of inseminations per calving, number of inseminations during last lactation and their age. Our results indicate that the intensity of CBHR is lowest in cows inseminated once or twice and younger animals, while, there were no differences between the groups when they were formed according to the number of inseminations per calving. Also, the intensity of CBHR was higher in older animals. Key words: PHA, cows, subfertility

Repeat breeding (RB) is still a significant problem in the reproduction of dairy cows. It is usually defined as a lack of conception following three subsequent artificial inseminations (AI) with no obvious symptoms of genital tract disease. A repeat-breeder is a cow which shows a reduced probability of conception, while all other factors are optimal (Casida, 1961), and thus requires more AI attempts to achieve pregnancy. If mated naturally, RB cows or heifers very often show improved reproductive results (Vukotič et al., 1982). The possible causes of this phenomenon are numerous and include delicate nutritional imbalance, subtle hormonal disturbances and immunological alterations. It was postulated that enhanced immunological reactivity to sperm or semen extender antigens might be one of the reasons for RB (Park and Hunter, 1977). In natural mating, an immune response to sperm antigens usually does not occur due to the low sperm and seminal plasma immunogenicity (Hogarth, 1982). Also, all body fluids, important for fertilization (seminal plasma, cervical mucus, follicular and uterine fluid) have immunosuppressive properties (Landers et al., 1994). In AI the chances for immunization of females to sperm antigens are enhanced because seminal plasma is diluted several times, thus reducing its very strong immunosuppressive effects (Lazarević, 1991; Lazarević et al., 1992) and new antigens originating from semen extenders are present. We have documented (Lazarević et al., 2003; Jačević and Lazarević, 2000; Jačević, 1998) by sperm agglutination and the indirect immunofluorescence method that bull spermatozoa differ in antigenicity if different semen extenders are used for semen preparation for AI. Therefore, it was possible to postulate that cows with high general
immunoreactivity (high responders) might also react to sperm and extender antigens and become repeat breeders.

One of the methods that are widely used in veterinary medicine to examine general immunological reactivity is the cutaneous basophile hypersensitivity reaction to phytohemagglutinin (PHA). PHA is an herbal lectin with mitogenic properties and usually serves in laboratory practice to induce nonspecific proliferation of mononuclear cells (Ekkel et al., 1995). If administered intradermally, PHA provokes a hypersensitive reaction characterized by enhanced skin thickness, erythema, indurations and swelling, accompanied with mononuclear cell accumulation. Histological examination of the skin at the site of PHA injection reveals massive accumulation of basophile cells and therefore this phenomenon was described as the cutaneous basophile hypersensitivity reaction or, alternatively, the PHA skin test. Moreover, PHA leads to changes in permeability of high endothelial venules, enabling easier passage of lymphocytes in tissues (Stedecker et al., 1977). The same authors postulated that the CBHR is a consequence of lymphokine production by stimulated mononuclear cells. The degree of reaction is highest 24 hrs after application (Me Corkie et al., 1980). This test has been used to investigate the influence of various stress factors on the immune response in various domestic animals (Ekkel et al., 1995; Wesley and Kelly, 1984; Kelley et al., 1982; Regnier and Kelley, 1981; Žikić, 2000; Lazarević et al., 2000). It was clearly documented that under intensive stress conditions the CBH reaction is significantly reduced. The PHA skin test is frequently used as an indicator of in vivo cellular immunity in calves (Kelley et al., 1982).

**Materials and methods**

Cutaneous basophile hypersensitivity reaction (CBHR): A total of 157 Holstein cows and 20 heifers were tested by intradermal application of 200 ug of PHA (INEP, Zemun). PHA was diluted in sterile phosphate buffered saline (PBS) pH 7.2, to reach a final concentration of 1 mg/mL and 200 uL of this solution was intradermally injected into the neck region. As a control, the same volume of sterile PBS was injected at a 15 cm distant site. Skin thickness (ST) was measured by cutimeter prior to administration of PHA or PBS and 24 hrs later. The magnitude of the reaction was calculated as the difference in double-fold skin thickness between the sites of PHA and PBS application according to the following formulas: 

$$D_{PHA} = ST_{Oh} - ST_{24h}$$

$$CBHR = D_{PHA} - D_{PBS} = ST_{Oh} - ST_{24h}$$

Basically, animals were divided into four groups according to the total number of previous artificial inseminations as follows: heifers, cows and heifers inseminated up to 5 times, cows inseminated 5.1-8 times and cows inseminated over than 8.1 times. Furthermore, the results obtained were analyzed considering the number of inseminations per calving (1 - 2, 2.1 - 3, 3.1 - 4, 4.1 - 5 and over 5.1 times), number of inseminations in the last lactation (0 - 3, 3.1 - 6 and over 6.1 times) and also according to the age of heifers and cows (less than 2 yrs, 2.1-4 yrs, 4.1 - 6 yrs. and over 6.1 yrs). Statistical analyses were performed after calculating mean values and standard deviations. The significance of the differences between mean values was estimated by LSD and Student's t test.
Results and discussions

Our results indicated differences in the degree of cutaneous basophile reaction to PHA (CBHR) in cows with different total numbers of artificial inseminations (Graph 1). These differences were statistically significant when 8HR results obtained on the animals that were not inseminated (virgin heifers) and cows inseminated more than 5 times were compared. We found the same level of significance when we compared the results obtained for the animals inseminated 0-5 and 5.1-10 times.

![Graph 1. CBHR in cows with different number of artificial inseminations (X±SD)](image)

When we sorted the results obtained for CBHR according to the number of AI needed for successful pregnancy we found no statistical differences between the experimental groups (Graph 2).

![Graph 2. CBHR in cows with different numbers of artificial inseminations per pregnancy (X±SD)](image)
Results of the CBHR in cows with different numbers of artificial inseminations during last lactation (X±SD) are presented on the graph 3. It is evident that significant statistical difference exist only between cows inseminated 0-3 times and 3.1-6 times and there is no regularity between analyzed parameters.

Graph 3. CBHR in cows with different numbers of artificial inseminations during last lactation (X±SD)

Finally, all cows included in this study were divided into four groups according to their age. It was documented that the younger animals (up to two years of age) had the lowest CBHR (Graph 4) and the differences between mean values were significant among this group and cows older than 4.1 years. We found no statistically significant differences between other groups of animals.

Graph 4. CBHR in cows of different age (X±SD)
After years of implementing artificial insemination procedures in cattle breeding it seems that some cows "resist" AI. Since one of the causes of this phenomenon might be an immunological barrier to conception, it was possible to postulate that cows with high general immunoreactivity (high responders) might also react to sperm and extend antigens and become repeat breeders. In order to check this hypothesis we have investigated the relationship one of the tests for estimating general cellular immune reactivity (CBHR) with the reproductive results of cows.

We have shown that the intensity of CBHR is significantly lower in heifers that were not inseminated and in cows inseminated 0-5 times compared to that in animals inseminated more than 5.1 times. However, we found no differences between the other experimental groups and therefore it is our opinion that the degree of reaction is probably more related to the age of the animals. This statement is confirmed by analyzing the same results sorted according to the age of the cows. In this case, we were able to demonstrate that younger animals (up to 3 years of age) also have a significantly lower intensity of skin hypersensitivity reaction to PHA. Moreover, when we sorted data according to the number of inseminations needed for successful pregnancy, we found no differences in CBHR among the experimental groups. It is, then, possible to conclude that there are differences in CBHR related to age. These results are in agreement with our previous findings obtained on a smaller group of heifers and cows (Lazarević et al. 2004).

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