

## MATERNAL ABILITY IN ALBINO RATS AND ITS EFFECT ON THE IMMUNE RESPONSE OF PUPS

BY

MERVAT, M. KAMEL\* and JAKEEN EI-JAKEE\*\*

\* Dept. Hygiene and Animal Ethology

\*\* Dept. Microbiology, Fac. Vet. Med., Cairo Univ.

Received 9/10/1993

### SUMMARY

A study was carried out to determine the influence of maternal ability in albino rats (*Rattus Norvegicus*) on the immune response (cellular immunity) of pups. Twenty multiparous females were used for the study. The dams were randomly arranged under normal environmental conditions. The following maternal behavioural parameters of dams were measured and recorded daily for 15 days following parturition; nest building, physical tactile contact, nursing and licking.

In addition the phagocytic activity (Percentage of phagocytosis) in pups was measured. The relative weights of thymus and spleen of pups were determined (Organ / body ratio).

The results indicated that; nest building, physical tactile contact, nursing (Time of nursing and crouching position) and licking were significantly & positively correlated with phagocytic percentage ( $p < 0.01, 0.05$ ) at the first 5 days and 15 days of pups' life. While retrieving not significantly correlated with phagocytosis. Concerning the relative weights of thymus and spleen of pups, the results declared that, there was no any correlation between them and the phagocytosis.

It was concluded that, proper maternity towards neonates might affect differentially the health, viability and immune protection against diseases of the pups.

### INTRODUCTION

In mammals the contacts between maternal and fetal circulation tend to be increasingly close due to the reduction of the number of cell layers separating them, where the close contact facilitates the transmission of IgG from the maternal to the fetal side in human fetus and in the rhesus relatively little antibody is transferred by the rat placenta (Sheldrake and Husband, 1985).

In suckling rats the epithelial cells along the small intestine display specific receptors for IgG to permit uptake and transcellular transport of IgG in milk, from the gut to the blood stream. Lymphocytes are absorbed by the intestine of the neonate rats by which immunity may confer from mother to its suckled young (Peppard et al., 1984 and Baintner, 1984).

The behaviour of the dam towards neonate affects the offspring's behaviour, temperament, body weight and viability and survival.

It is well accepted that good mother ability especially suckling enhances the ability of the newborns to be protected against infection. It is believed that the aetiology of several neonatal diseases and mortalities are complicated by the maternal behaviour malfunction of the dam towards the neonate.

It would be interesting to study somethings on the maternal ability and the cellular immunity of neonate, so, this trial was carried out to study the correlation between the important parameters of

maternal ability in rats and some aspects of the immune response of the neonates.

## MATERIAL AND METHODS

### Animals:

Albino rats (*Rattus Norvegicus*) were used in this study. Twenty multiparous females were used, each with an average weight 250-300 mg.

Femals rats were bred by placing a male in a cage with femals, over night. Pregnancy was insured by copulatory plugs.

### Housing and maintenance:

Each female was housed in cage (40 x 20 x 18 cm) and clean sawdust was used as bedding. All females were maintained under 12:12 hr reversed day/night cycle and under a relatively ambient temperature of 20-25°C. Relative humidity was between 50-70 %. Animals were fed rat chow and water ad libitum.

### I. Behavioural measurements:

Females in each cage with their pups were observed daily for 15 days following parturition.

Two times of observation were chosen 8 A. M. and 2 P. M., for each period 15 minutes were taken in observation (30 minutes for each dam daily), the following parameters were observed and recorded according to McIver and Jeffrey (1967).

#### (1) Nest building

Nest building behaviour, resulting in an organized nest, arises shortly before parturition and continues for the first two weeks of litter care, then declines and disappears.

Nest building were classified into four grades, excellent (+3), good (+2), fair (+1) and poor (0).

#### (2) Physical contact

Based on five scales, four points if the dam was in contact with all pups (excellent), three points, if the dam was in contact with all pups except one or two (good), two points if the dam was in contact with three pups (fair), one point if the dam was in contact with only one pup or two pups (poor) and no points if she was not in contact with any of

them.

#### (3) Retrieving

After delivery the female picks up and carries to the general zone of parturition the newly born fetuses that rolled to a distance from it.

Points were given as follows, four points if the pups were arranged in one pile (excellent), three points if the pups were arranged in a pile except for a single stray (good), two points, if the young were grouped into two piles, one point if there was one pile and two or three strays (fair) and no points if the pups were scattered.

#### (4) Nursing

Nursing behaviour was recorded on the bases of four scores. Score four (excellent), the dam is in nursing posture, score three (good), the dam was in partial nursing posture, score two (fair) the dam was not in nursing posture, but was relaxed or sleeping on her side or she was in partial nursing posture, but actively eat or drink and score one (poor or non), the dam was not in nursing posture and moving about the cage.

#### (5) Licking

The dam vigorously licked her pups after parturition. During observation, the duration of licking was recorded in minutes.

Licking was recorded on the bases of four scores: Excellent, the dam spent the whole time of observation licking her pups (30 min), good the dam spent about 15 min licking the pups (half time of observation), fair, the dam spent about 5 to 15 minutes in licking while score poor or non if the dam failed to lick her pups.

## II. Evaluation of cellular immunity of pups:

### \* Blood samples

Blood samples were collected from the pups two times, at the 5th day and 15th day of life.

The samples collected in sterile vial containing heparin for lymphocytes separation.

### \* Media

- Lymphocytes separation medium (sigma, U. S. A.) Ficoll hypaque was used for separation of

## Maternal ability

mononuclear leukocytes from peripheral blood.

- RPMI 1640 tissue culture medium used in the lymphocytes preservation.
- Sterile fetal calf serum (Gibco limited, U. K.).
- Sabouraud's dextrose agar for cultivation of *Candida albicans* to be used for phagocytic activity assay.

### \* Reagents

- Heparin solution ampules (5000 i. u.) were used as anticoagulant.
- Phosphate buffered solution PBs 0.02 M, PH 7.2

### \* Stains

- Giemsa stain for staining phagocytic cells.

### \* Techniques

Isolation and cultivation of peripheral blood mononuclear phagocytic cells (Antley and Hazen, 1988).

The phagocytic activity was determined by adding 1 ml of  $1 \times 10^6$  yeast cells/ml to the adherent phagocytic cells.

After incubation for 1 hour, cover slides were used several times and stained with Giemsa stain (Carter, 1973). The following equation (Richardson & Smith, 1981) was used for the calculation of the phagocytic activity: percentage of phagocytosis

$$= \frac{\text{Number of investing phagocytes}}{\text{Total number of phagocytes}} \times 100.$$

1. Relative weight of thymus and spleen (Organ/body ratio):

The relative weight of thymus and spleen was determined according to Giambone (1989), where the thymus and spleen of each pup were removed and their weights and total body weight of the same pup were determined.

Ratio of organ to body weight was calculated according to the following equation:-

Organ/body ratio

$$= \frac{\text{weight of organ (thymus or spleen)}}{\text{Total body weight}} \times 100$$

### Statistics:

All the obtained results were subjected to "correlation coefficient" test.

## RESULTS

### (1) Maternal parameters:

Table (1): Incidence of maternal parameters in female rats

Maternal parameters	Dams showed maternal parameters	
	Number	%
* Nest building:		
- Non or poor	4	20
- Fair	2	10
- Good	8	40
- Excellent	6	30
* Physical contact -		
Non or poor	6	30
- Fair	4	20
- Good	8	40
- Excellent	2	10
* Retrieving		
- Non or poor	2	10
- Fair	6	30
- Good	4	20
- Excellent	8	40
* Nursing		
- Non or poor	4	20
- Fair	6	30
- Good	8	40
- Excellent	2	10
* Licking		
- Non or poor	6	30
- Fair	2	10
- Good	10	50
- Excellent	2	10

Table (1), shows the incidence of the different parameters of the maternal behaviour:-

#### \* Nest building

20 % of dams showed non or poor nest building, while the other females (80 %) constructed their nests in a manner arranged as following, 10 % fair nest building, 40 % good nest building and 30 % excellent nest building.

#### \* Physical contact

With respect to the expression of physical contact, 30 %, 20 %, 40 % and 10 % of the

females respectively showed physical contact toward their neonates as the following manner, non or poor, fair, good and excellent.

**\*Retrieving**

40 % of the dams grouped their pups into one pile (excellent dam), while 20 % were good dams, 30 % fair dams and 10 % of dams failed to group their pups in the nest.

**\* Nursing behaviour**

The results indicated that 30 %, 40 % and 10 % of the female rats respectively showed fair, good and excellent nursing posture toward their pup. While 20 % of the dams failed to suckle their pups.

**\* Licking behaviour**

Regarding to the time spent by the dam licking their pups, it was found that, 10 %, 50 % and 10 % of the dams respectively considered fair, good and excellent dams in spending more time licking the pups. While 30 % of the dams considered poor in their expression of licking toward their pups or failed to lick the pupung.

**(II) Immune response of pups Vs. maternal variables**

Table (2): Correlation between phagocytosis percent of neonates and maternal variables (nest building, physical contact, retrieving, nursing and licking) performed by female rats.

Phagocytosis Vs.	Correlation coeff.	
	5th day of life	15th day of life
* Nest building	+ 0.91*	+ 0.42
* Physical contact	+ 0.82*	+ 0.70*
* Retrieving	+ 0.37	- 0.09
* Nursing:		
Time of nursing	+ 0.69**	+ 0.40
Crouching position	+ 0.69**	+ 0.55**
* Licking	+ 0.77*	+ 0.58**

\* Significant P < 0.01  
 \*\* Significant P < 0.05

Regarding the correlation between the different maternal parameters and the immune response of the pups represented by phagocytosis percent, table (2) shows that::

**\* Nest building Vs. phagocytosis %:**

Nest building was significantly positive correlated with percent of phagocytosis at the first five days

of life ( $r = 0.91, < 0.01$ ) while at 15 days of life not significantly correlated ( $r = 0.42, p < 0.05$ ).

**\* Physical contact Vs. phagocytosis%:**

The correlation coefficient between the time of physical contact which exerted from the dam to its neonates and the phagocytosis percent was significant at the first 5 days and 15 days of life (respectively  $r = 0.82$  and  $r = 0.70, p < 0.01$ ).

**\* Retrieving Vs. phagocytosis %:**

The correlation between retrieving and phagocytosis % not significant.

**\* Nursing Vs. phagocytosis %:**

Time of nursing was found to be significantly and positively correlated with phagocytosis percent at the first five days of life ( $r = + 0.69, P < 0.05$ ), while it was non significant at 15 days of life ( $r = + 0.4$ ).

On the otherhand, crouching position was significantly correlated with phagocytosis percent at both 5th and 15th day of life ( $r = + 0.69$  and  $+ 0.55$  respectively,  $P < 0.05$ ).

**\* Licking Vs. phagocytosis %:**

Licking was significantly correlated with phagocytosis percent at both 5th and 15th day of life wherer =  $+0.77$ , and  $r = + 0.58$  respectively  $P < 0.01$  and  $P < 0.05$ .

**Relative weights of thymus gland and spleen of pups:**

The average relative weights of thymus gland were 3.478 and 7.203 respectively at the day 5 and 15 of age.

Regarding to the relative weights of spleen, it was found that 2.144 and 3.275 respectively at day 5 and 15 of age of pups. The results declared that, there was no any correlation between the relative weights of both thymus gland and spleen and the phagocytosis percent.

**DISCUSSION**

At least during the first week of neonates life, the good mother ability in female rat is to keep their young in better order. From the study, the mother

ity or maternal behaviour performed by the dam towards the neonates, not only important for the establishment and maintenance of postpartum maternal responsiveness in the dam, but also it would play an important role in the immune response of the neonates, where it may enhance the ability of the newborn to be protected against infections. In addition, it is believed that the aetiology of several neonatal diseases and syndromes are complicated by the maternal behaviour malfunction of the dam towards the neonates.

This study was designed to investigate the relationship between mother abilities of female rats performing towards the neonates and one of the most important essentials of survival and protection against neonatal diseases and syndromes.

On one hand, nest building, physical contact, nursing, nursing and licking scores are suitable variables for the maternal behaviour in female rats (Denenberg et al., 1969; Hafez, 1970; Grotta & Wolf, 1974 and Ehret & Bernecker, 1986). On the other hand, phagocytosis index is one of the reliable variables for determination of the immune state of individuals (Jawetz et al., 1980; Brown, 1984; Southwick & Stossel, T. P. 1986). Phagocytosis render various microbes non virulent either independently or by interaction with the alternative pathway of complement and the defensive activities of humoral and cell-mediated immunity are largely promoted by phagocytosis and the complement system. B-cells and macrophages function as the scouts, hunting for foreign antigens, when a macrophage encounters an antigen, it presents the antigen via its MHC proteins to a helper T-cell that recognizes the complex of MHC and antigen and activate the cells that attack and destroy the invader (Watson et al., 1992).

Physical contact is needed for direct contact with the dam for the establishment of post-partum maternal responsiveness and survival of the neonates (Findley, 1966 and Jakubowski & Wolf, 1986).

Physical contact also could make phagocytosis more efficient by stimulation the circulation of the

neonate and consequently enhancing the absorption of coated bacterial surface with antibodies from the intestine of the neonate. The coated bacterial surface with the antibodies from the intestine of the neonate. The coated bacterial surface with the antibodies facilitates the uptake of bacteria by phagocytes (Jawetz et al., 1980).

Denenberg et al. (1969) reported that nest has functional value in that young were packed together in the nest, thus helping to maintain body warmth and enhancing the survival value of the species. These altricial species require more protection since they are born relatively naked, and the mother sitting above the young to form a heated roof for them (Payne, 1976). The positive and strong correlation between nest building and phagocytosis percent ( $r = + 0.91, P < 0.01$ ) indicates that dams who build good nests will give a high percent of alive and healthy pups with good immune response especially during the first week of life.

Licking the neonates by their dam during suckling and also the period of suckling play an important role in vital processes of the neonates body for stimulation of urination, defecation and circulation (Rosenblatt and Lehrman, 1963). The results of the present study indicate strongly that neonates which receiving more and good licking from their dam, had high percentage of phagocytosis during the first two weeks of life. Peppard et al. (1984) and Baintner (1984) indicated that lymphocytes are absorbed by the intestine of the neonates rat by which immunity may confer from mother to its suckled young.

Concerning the strong and positive significant correlations between the phagocytosis percent and both time of nursing and the degree of crouching position that performed by the dams towards their neonates, it indicates that dams who consume long time for suckling their neonates with adequate crouching position, to facilitate the process of suckling, will give healthy and strong pups. Moltz (1975), described the mother crouching over the young in such a way to expose her mammary region and adjusts her posture, allowing attachment with nipples and remain entirely passive to sustain attachment.

Retrieving appears regularly in response to the general stimulus of a pup located outside the nest and declines between the 12th and 16th days after parturition (Rosenblatt and Lehrman, 1963). There was a positive but non significant correlation between retrieving and phagocytosis percent at the first week of life.

On the otherhand the correlation decreased with progressing in age, it changed to a very weak one at the second week of neonates life.

In conclusion, this study may explain the possibility that, early life maternity towards neonates might affect differentially the health and viability of neonates (Rosenblatt & Lehrman; 1963. Moltz; 1975, Ehret & Bernecker; 1986 and Jakubowski & Terkel; 1986) as well as the protection against several neonatal diseases. Evidence of our results has been gained to support the theory, where dams who show adequate nest building, physical tactile contact with pups, crouching over pups to allow the attachment with nipples and suckle them for long time as well as licking their pups during nursing, will give a high percent of viable pups with good immune protection against diseases.

## REFERENCES

- Antley, P. P. and Hazen, K. C. (1988): Role of yeast cell growth temperature on candida albicans virulence in mice. *Infection and Immunity* 66; 2884-2889.
- Baintner, K. and Kocsis, G. (1984): Occurrence and some characteristics of the immunoglobulin G receptors of the small intestinal mucosa in-vitro experiments. *Acta Vet. Hung.* 32 (3-4): 181-186.
- Browry, T. R. (1984): *Immunology simplified*, 2nd Ed. Oxford University Press. Walton Str., Oxford OX<sub>2</sub> 6DP.
- Carter, G. R. (1973): *Diagnostic procedures in veterinary microbiology* 2nd Edition. 301-327, East Lawrence, Springfield, Illinois, U. S. A.
- Denenberg, V. H., Taylor, R. E. and Zarrow, M. X. (1969): Maternal behaviour in the rat: An investigation and quantification of nest building, *Behaviour*. 34: (1-2), 1-16.
- Ehret, G and Bernecker, C. (1986) *Low-frequency communication by mouse pups (Mus musculus) Wriggling calls release maternal behaviour*, *Anim. Behav.* 34 821-830
- Findley, A. C. R. (1966): Sensory discharges from mammary glands. *Nature, London.*, 211, 1183-1184
- Giambone, J. G. (1989): Review in *immuno suppression in poultry Momentum technical information for agent 1: 5-9.*
- Grota, L. J. and Ader, R. (1974): Behaviour of lactating rats in a dual-chambered maternity cage. *Horm. Behav.* 275-282.
- Hafez, E. S. E. (1970): *Reproduction and feeding techniques for laboratory animals*. Lea and Febiger, Philadelphia, PA.
- Jakubowski, M. and Terkel, J. (1986): Establishment and maintenance of maternal responsiveness in post-parturient Wistar rats. *Anim. Behav.*, 34: 256-262.
- Jawetz, E., Melnick, J. L. and Adelberg, E., A. (1976): *Review of Medical Microbiology*. Lange Medical Publication Los Altos, California.
- McIver, A. H. and Jeffrey, W. E. (1967): Strain differences in maternal behaviour in rats. *Behaviour*, 28: 210-216
- Moltz, H. (1975): Maternal behaviour: Some hormonal, neural and chemical determinants in: *The behaviour of domestic animals* (Ed. by E. S. E. Hafez) 3rd ed., pp. 146-170, Bailliere Tindall, London.
- Payne, A. (1976): *Social behaviour in vertebrates*. Heinemann Educational Books.
- Peppard, J. V., Jackson, L. E., Hall, J. G. and Robertson, D. (1984): The transfer of immune complexes from the lumen of the small intestine of the blood stream in suckling rats. *Immunology* 53 (2): 385-394.
- Richardson, M. D. and Smith, H. (1981): Resistance to virulent and attenuated strains of *C. albicans* to intracellular killing by human and mouse phagocytes. *Infect. Dis.*, 144, 557-565.
- Resenblatt, J. S. and Lehrman, D. S. (1963): Maternal behaviour of the laboratory rat. In: *Maternal behaviour in mammals* (Ed. by H. L. Rheingold). PP. 8-57. New York; John Wiley.
- Sheldrake, R. F. and Husband, A. J. (1985): Intestinal uptake of intact maternal lymphocytes by neonatal rats and lambs. *Res. Vet. Sci.* 39 (1): 10-15.
- Southwick, F. S. and Stossel, T. P. (1986): Phagocytosis. In: *Manual of clinical laboratory immunology* (3rd Ed.) by Noel R. Rose, Herman Friedman and Washington, D. C., John L. Fahay A. S. for microbiology.
- Watson, J. D., Gilman, M. Witkowski, J. and Zoller, M. (1992) *Recombinant DNA* 2nd Ed., W. H. Freeman and Company New York.