Effect of Anti-Placenta Antibody on the Fertilization and Early Pregnancy in the Mouse

Miyano, Takashi
Kato, Seishiro
Kanda, Sunao
Iritani, Akira

(Citation)
神戸大学農学部研究報告,18(1):119-124

(Issue Date)
1988-01

(Resource Type)
departmental bulletin paper

(Version)
Version of Record

(JaLCDOI)
https://doi.org/10.24546/00200495

(URL)
https://hdl.handle.net/20.500.14094/00200495

神大農研報 (Sci. Rept. Fac. Agr. Kobe Univ.) 18:119−124, 1988

EFFECT OF ANTI−PLACENTA ANTIBODY ON THE FERTILIZATION AND EARLY PREGNANCY IN THE MOUSE

Takashi MIYANO *, Seishiro KATO *, Sunao KANDA * and Akira IRITANI **

(Received for publication on August 10, 1987)

Abstract

Expression of placental specific antigen(s) in unfertilized mouse eggs and early embryos, and the effect of the antibody on the fertilization and early pregnancy in the mouse were investigated. The antisera against mouse placenta were obtained from rabbits 10 days after the six injections of placental extracts with Freund's complete adjuvant. Anti-placenta antibody was purified from non-absorbed and kidney- and liver-absorbed antisera. In indirect immunofluorescent antibody assay, unfertilized eggs and early embryos were treated with anti-mouse placenta antibody and FITC-conjugated anti-rabbit γ-globulin antibody. The antigen(s) similar to the mouse placental specific antigen(s) were expressed on the cell surface of 2-cell embryo and also on 4-cell embryo, morula and blastocyst. By contrast, no fluorescence was detected on the unfertilized egg and 1-cell embryo, suggesting that they do not have antigens reacting with the anti-placenta antibody. In the next experiment, the effect of the antibody on the fertilization and early pregnancy was examined by passive immunization. Non-absorbed antibody decreased the fertilization rate but kidney- and liver-absorbed anti-placenta antibody had no effect on the fertilization and early pregnancy (days 3−5 of gestation). These results show that the ovulated eggs do not have the placental specific antigen(s) and thus the anti-placenta antibody has no effect on the fertilization. Although the early embryos have the placental specific antigen(s), the antibody has no effect on their development *in vivo*. This suggests that the early embryos may have specific protective mechanisms against the antibody.

Introduction

The fetuses of mammals are immunologicaly allografts, but they are not rejected by maternal immune system during pregnancy. The mechanism involved in the maintenance of pregnancy has not been clear. It has been suggested that placenta may be the functional interference between mother and fetuses[6,10]. The placenta contains many specific antigens which are not detected in other tissues[1]. Although their functions are still unknown, these placental specific antigens may play a role for maintenance of pregnancy. In the previous study[8], we detected three specific antigens in mouse placenta on day 14 or 15 of gestation, and found that the anti-mouse placenta antibody produced in rabbits interrupted the pregnancy on days 7 − 10 of gestation. The present study was undertaken to examine the expression of the placental specific antigens in the unfertilized eggs and early embryos with indirect immuno-

* Laboratory of Animal Reproduction

** Department of Animal Science, College of Agriculture, Kyoto University

fluorescent antibody assay, and the effects of antibody against placental specific antigens on the fertilization and early pregnancy in the mouse were also examined.

Materials and Methods

1. Preparation of Anti-Mouse Placenta Antibody

The antiserum against mouse placenta was produced in Japanese White rabbits. Mouse placentae were collected on day 14 or 15 of gestation from JCL – ICR mice. They were washed with phosphate buffered saline (PBS, pH 7.3) and homogenized in PBS with glass and ultrasonic homogenizers (Nihon Seiki Co.). The homogenate was centrifuged at 10,000×g for 30 min, and the supernatant was emulsified with an equal volume of Freund's complete adjuvant. The emulsion was injected six times into female rabbits at an interval of 10 days. Ten days after the last injection the blood was collected, clotted at room temperature, and antisera were obtained by centrifugation at 1,500×g for 20 min. An aliquot of antisera was absorbed with mouse kidney and liver homogenates to react with only placental specific antigens, since they reacted with not only placenta but other tissue antigens[8]. The absorbed and non-absorbed antisera were decomplemented at 60°C for 30 min and γ-globulin fraction was isolated by salting out (46% of saturated ammonium sulfate[4]) and gel chromatography (Sephadex G-100, 3.2×45 cm). The resulting antibody solution (12 mg protein/mℓ) was stored at −20°C before use.

2. Indirect Immunofluorescent Antibody Assay

The expression of the placental specific antigens in the unfertilized mouse eggs and early embryos was investigated with indirect immunofluorescent antibody technique. Female mice were induced to superovulate by an intraperitoneal injection of pregnant mare's serum gonadotropine (PMSG, 5 iu) followed 48 hr later by a similar injection of human cholionic gonadotropine (hCG, 5 iu). After 15 hr of hCG injection, unfertilized eggs were collected from oviducts. After 24–96 hr of hCG injection, early embryos at various stages of development were collected from the oviducts and uteri of female mice caged overnight with males. The unfertilized eggs and early embryos were washed with PBS and dehydrated with ethanol quickly. They were treated with liver- and kidney-absorbed antibody solution at 37°C for 20 min and washed with PBS thoroughly. Then they were treated with diluted FITC-conjugated goat anti-rabbit IgG antibody (1 : 80, Miles Laboratories, InC.) for 20 min. They were washed well and examined under fluorescent microscope (Nicon fluorescence microscope). Control preparation were treated by the same manner with placenta-absorbed antibody solution.

3. Passive Immunization

The effect of anti-placenta antibody on fertilization was examined by passive immunization based on the method developed for the test of inhibitory action of anti-zona pellucida antibody on fertilization[11]. Mature female mice (JCL – ICR) were induced to superovulate as described above. After 5 hr of PMSG injection, each mouse received an intraperitoneal injection of 0.3 mℓ non-absorbed or absorbed antibody solution. Control animals received IgG solution form non-immunized rabbits as the same manner. The female mice were mated with males and 21–22 hr after hCG injection the eggs were collected from oviducts. The eggs were mounted *in toto*, stained with 0.25% lacmoid, and examined for fertilization[3]. Eggs which had male and female pronuclei in the vitellus were considered to be undergoing fertilization. Eggs which had fragmented or degenerated vitellus were considered to be abnormal. Effect of anti-placenta antibody on early pregnancy in the mouse was also examined by passive immunization. Female mice were mated and the day on which vaginal plugs were

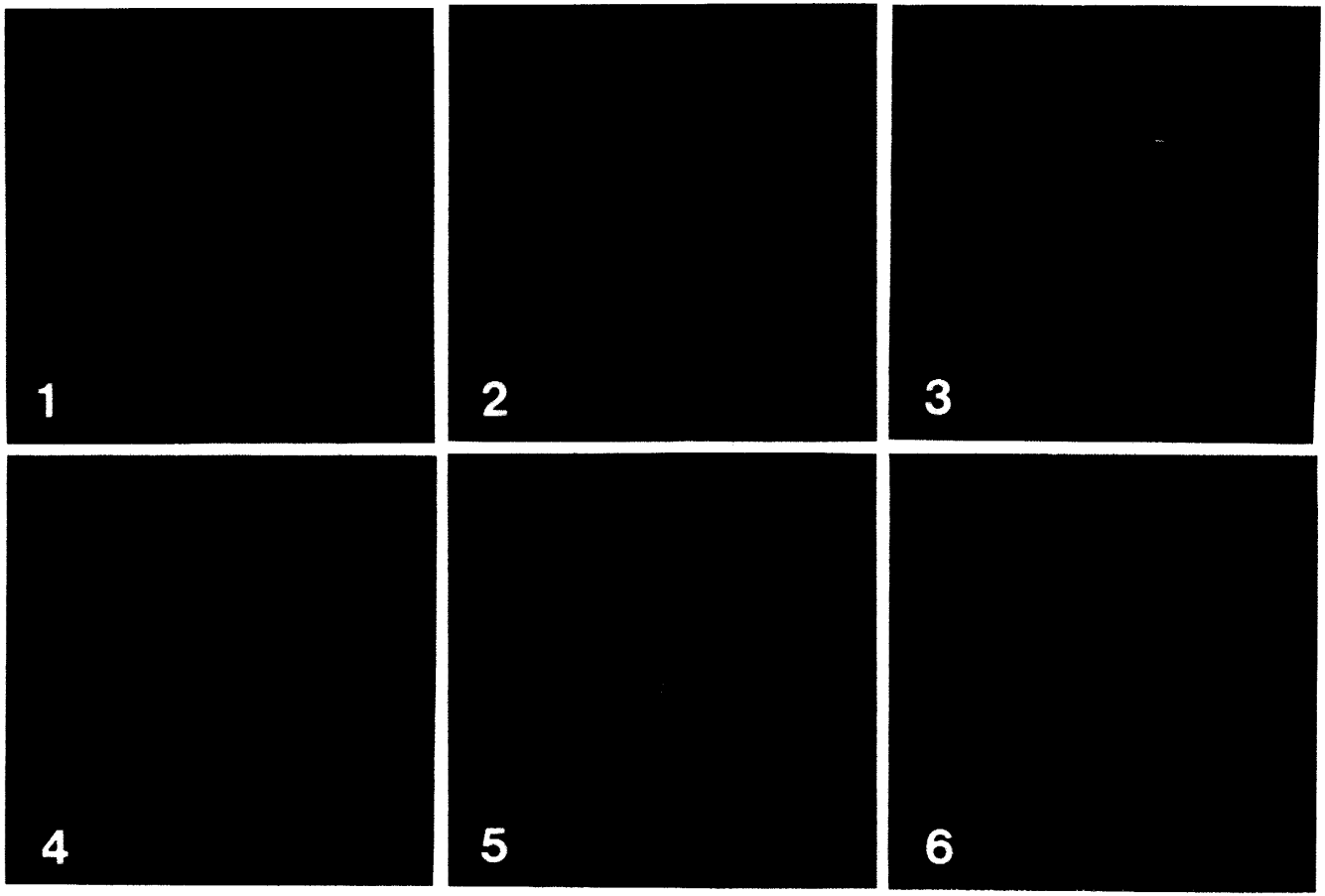

Plate 1

Immunofluorescent microphotographs of mouse unfertilized egg and embryos at various stages of development. They were treated with anti-mouse placenta antibody and FITC-conjugated anti-rabbit IgG antibody.

Fig. 1 Unfertilized egg Fig. 2 Fertilized egg Fig. 3 2-cell embryo
Fig. 4 4-cell embryo Fig. 5 Morula Fig. 6 Blastocyst

Table 1 Effect of anti-mouse placenta antibody on the fertilization in the mouse

Treatment+	No. of mice mated	No. of eggs examined	No. of eggs undergoing fertilization (%)	No. of abnormal eggs (%)
Normal rabbit IgG	5	85	72 (84.7)	12 (14.1)
Anti-placenta antibody	6	146	98 (67.1)*	43 (29.5)*
Anti-placenta antibody (absorbed with kidney & liver)	6	113	100 (88.5)	13 (11.5)

+ Single injection of 0.3 ml antibody solution was made into each mouse 5hr after injection of PMSG.
* Statistical difference from control ; $P < 0.01$ (x^2 test)

Table 2 Effect of anti-mouse placenta antibody on the early pregnancy in the mouse

Treatment*	No. of mice mated	No. of pregnant mice (%)	Total no. of fetuses	Total no. of abnormal fetuses (%)
Normal rabbit serum	9	7 (78)	73	4 (5.5)
Anti-placenta antibody (absorbed with kidney & liver)	11	8 (73)	77	5 (6.5)

* Three injections of antibody solution, 0.6 ml in total were made into each mouse on days 3, 4 and 5 of gestation.

observed was designated day 1 of gestation. On days 3 – 5, each mouse received intraperitoneally with 0.2mℓ of kidney- and liver-absorbed antibody solution daily. On day 14 of gestation, mice were killed and examined for the interruption of pregnancy. Control animals received nonimmunized rabbit serum as described above.

Results

The expression of mouse placental specific antigens on unfertilized eggs and early embryos was examined with indirect immunofluorescent antibody technique. No fluorescence was observed either in unfertilized eggs or in 1-cell embryos (Plate 1, Figs. 1 and 2). On the other hand, weak immunofluorescence was observed on the surface of 2-cell embryo (Plate 1, Fig. 3), and also on the 4-cell embryo, morula and blastocyst (Plate 1, Figs. 4, 5 and 6). At any stages, no fluorescence was observed on the zona pellucida. These results suggested that the antigen(s) similar to the mouse placental specific antigen(s) were expressed as early as 2-cell stage embryo.

Table 1 summarized the effect of anti-mouse placenta antibody on the fertilization after passive immunization. In the control mice received non-immunized rabbit IgG, 72 eggs from 85 were fertilized. Non-absorbed antibody inhibited the fertilization significantly. But fertilization rate of the eggs from the mice received kidney- and liver-absorbed antibody was similar to that in the control group. Abnormal eggs were increased by the treatment with non-absorbed antibody, but absorbed antibody has no effect on the incidence of abnormal eggs.

Table 2 shows that the inhibitory effect of absorbed anti-placenta antibody on early pregnancy was not observed. The incidence of abnormal fetuses was not different between control and experimental animals.

Discussion

The present study made it clear that mouse unfertilized eggs and 1-cell embryos did not have the placental specific antigen(s), while the antigen(s) were expressed on the cell surface of as early as 2-cell embryo (Plate 1). We have shown that the antisera against mouse placenta on day 14 or 15 of gestation react with three placental specific antigens after absorption with mouse kidney and liver homogenates[8]. Although the absorbed antibody reacted with the surface antigen(s) of early embryos, it is uncertain which one of the three specific antigens is responsible.

There are several reports concerning the specific surface antigens of mouse early embryos. WILEY and CALARCO[12] detected the placental specific antigen(s) on the cell surface of early embryos using the absorbed anti-placenta antiserum. According to them, the antiserum reacted with the ovarian and ovulated eggs and embryos throughout preimplantation development. These results differ from ours in recognizing the specific antigen(s) on unfertilized eggs or 1-cell embroys. MOSKALEWSKI and KOPROWSKI[9] detected oocyte specific antigen(s) on the cell surface of oocytes and early embroys. MENGE and FLEMING[7] reported that ovary absorbed anti-mouse sperm antiserum reacted with the embryos at the 4-cell stage to the blastocyst. Since these two reaction patterns of the antisera were different from our results, it seems that we detected the different angiten(s) from the oocyte or sperm specific antigens.
or sperm specific antigens.

In the passive immunization test, the absorbed anti-placenta antibody had no effect on the fertilization (Table 1). This may be due to the absence of the placental specific antigen(s) on the surface of ovulated eggs as shown in the indirect immunofluorescent antibody assay.

The anti-placenta antibody did not influence the early development of mouse embryos *in vivo* (Table 2). Since placental specific antigen(s) were present on the surface of embryos in this stage of development, the antibody must interact with the antigen(s). The reason why no effect was observed is not clear, but early embryos before implantation might have a defensive mechanism against maternal immune attack. JAMES[5] suggested that the zona pellucida deffended the embryos from the maternal immune system. However, this seems unlikely in the present case, since hatched embryos are exposed to the antibody.

CALARCO and BANKA[2] examined the effect of antiserum to an embryonal carcinoma cell line on the early development of mouse embryos. They detected the same antigens on the surface of preimplantation embryos, but they found no effect of antiserum on the development of embryos *in vitro*. They suggested that the antigens might loosely associate with the membrane or shed in the response to the antibody binding. The same possibility cannot be excluded in the case of the placental specific antigen(s) on the surface of embryos.

References

1) BOHN, H.: *Arch. Gynäk.*, **210**, 440–457, 1971.
2) CALARCO, P. G. and BANKA, C. L.: *Biol. Reprod.*, **20**, 699–704, 1979.
3) CHANG, M. C.: *J. exp. Zool.*, **121**, 351–382, 1952.
4) HAMASHIMA, Y. and KYOGOKU, M.: *Immunohistology*, 3rd ed., p69–75, Igaku Shoin Ltd., Tokyo, 1974.
5) JAMES, D.A.: *Transplantation*, **8**, 846–851, 1969.
6) KIRBY, D. R. S., BILLINGTON, W. D., BRADBURY, S. and GOLDSTEIN, D. J.: *Nature*, **204**, 548–549, 1964.
7) MENGE, A. C. and FLEMING, C. H.: *Develop. Biol.*, **63**, 111–117, 1978.
8) MIYANO, T., SATO, E. and IRITANI, A.: *Jap. J. Anim. Reprod.*, **26**, 94–97, 1980.

9) MOSKALEWSKI, S. and KOPROWSKI, H.: *Nature,* **237**, 167–168, 1972.
10) SIMMONS, R. L. and RUSSELL, P. S.: *Ann. New York Acad. Sci.,* **99**, 717–732, 1962.
11) TSUNODA, Y. and CHANG, M. C.: *J. Reprod. Fert.,* **54**, 233–237, 1978.
12) WILEY, L. M. and CALARCO, P. G.: *Develop. Biol.,* **47**, 407–418, 1975.

マウスの受精および初期妊娠過程に及ぼす抗胎盤抗体の作用

宮野　隆・加藤征史郎・苅田　淳・入谷　明

要　約

マウス未受精卵および初期胚における胎盤特異抗原の出現を調べるとともに，抗胎盤抗体の受精および初期妊娠過程に及ぼす作用を検討した。

マウス胎盤抽出液をフロイント完全アジュバントとともに6回にわたってウサギに免疫注射し，最終免疫10日後にウサギより抗マウス胎盤抗血清を得た。未吸収抗血清および腎臓・肝臓吸収抗血清より抗胎盤抗体を精製し，この抗体を用いてマウスの未受精卵および初期胚における胎盤特異抗原の出現を間接蛍光抗体法で調べた。その結果，胎盤特異抗原は未受精卵および1細胞期の胚には存在しないが，2細胞期以後の胚の細胞質表面に出現することが明らかとなった。また，抗胎盤抗体をマウスに受動免疫することによって，抗胎盤抗体の受精および初期妊娠過程に及ぼす作用を検討したところ，未吸収抗胎盤抗体は受精率を低下させるが，腎臓・肝臓吸収抗体は，受精および初期妊娠過程（妊娠3～5日）に対して作用を及ぼさないことが明らかとなった。

これらの結果から，排卵卵子は胎盤特異抗原を有しないため，抗胎盤抗体は受精に対しては作用しないことが明らかとなった。また，初期胚は胎盤特異抗原を有しているにもかかわらず，抗体の作用を受けないことから，抗体に対する特殊な防御機構を有していることが推察された。