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Ammonia Concentrations In Different Embryonic Developmental Stages In Wistar-Imamichi Rats In Vivo

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Abstract

Background: *Ammonia is a by-product of amino acid metabolism and has been shown to exert deleterious effects on embryonic development under experimental conditions. Despite this, little is known about the physiological regulation of ammonia metabolism during normal embryonic development in vivo. This study aimed to investigate developmental changes in ammonia concentrations within the maternal fetal environment during gestation.*

Materials and Methods: *Pregnant Wistar-Imamichi rats (220–260 g) were sacrificed at gestational days 10, 12, 14, 16, 18, and 20. Ammonia concentrations were measured in embryonic fluid, placenta, maternal serum, and maternal and fetal kidneys using the catalyzed indophenol colorimetric reaction, with absorbance measured spectrophotometer at 625 nm.*

Results: *Ammonia concentrations showed significant gestational stage dependent variations across all tissues examined. Placental and maternal serum ammonia levels increased significantly from day 10 to day 16, reaching peak values at mid gestation, followed by a marked decline toward late gestation. In contrast, ammonia concentrations in both maternal and fetal kidneys decreased progressively with advancing gestation and remained relatively stable after mid-gestation. No significant differences were observed between maternal and fetal kidney ammonia concentrations at corresponding developmental stages. Embryonic fluid ammonia levels exhibited a consistent decline from mid to late gestation, suggesting improved embryonic regulation of nitrogenous waste as development progressed.*

Conclusion: *These findings demonstrate that ammonia metabolism and clearance are dynamically regulated during gestation and highlight a potential window of increased metabolic sensitivity at mid gestation. The results underscore the importance of ammonia homeostasis in placental function and fetal physiology and provide insight into normal embryonic metabolic regulation in vivo.*

Keywords: *Ammonia, Embryonic development, Placenta, Kidney, Rat*

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I. Introduction

The term placenta refers to any combination of embryonic and maternal tissues that serves as the medium for physiological exchange between the mother and the developing offspring. It is formed through the integration of the blood-rich internal uterine lining with specialized extra-embryonic membranes. Living organisms excrete excess nitrogen produced during amino acid metabolism in one of three principal forms. Many aquatic animals excrete nitrogen directly as ammonia. In terrestrial environments, where water availability is limited, evolutionary adaptations have led to the conversion of ammonia into less toxic nitrogenous waste products that require less water for excretion. Ammonia, a by-product of protein metabolism, has been shown to reduce the viability of mouse embryos [1, 2]. Elevated ammonia concentrations have also been reported to decrease blastocyst cell numbers, reduce implantation rates, retard fetal growth, and increase the incidence of exencephaly following the transfer of cultured embryos into the uteri of surrogate mothers [3].

The accumulation of ammonium in embryo culture systems is primarily attributed to the spontaneous degradation of glutamine under standard culture conditions for pre-implantation embryos [4]. Several studies have suggested that ammonium present in culture media exerts deleterious effects on fetal development following embryo transfer in mice [5, 6, 7]. Previous reports from our laboratory demonstrated that ammonia

accumulation in culture media can be reduced by supplementation with amino acids, dipeptides, selenium, and vitamin E, which play important roles in porcine embryo development [8, 9].

The mechanisms underlying ammonia accumulation in ovarian follicles and the variation in ammonia concentration among follicles of different sizes have been described previously [10]. Higher ammonia concentrations in small follicles may reflect increased metabolic activity during early follicular development, as well as higher protein content within these follicles [11, 12, 13]. Numerous animal studies have demonstrated that the addition of exogenous ammonium to embryo culture media adversely affects embryonic development, pregnancy outcomes, and fetal growth [3, 10, 5, 7, 14]. Embryos appear to be particularly sensitive to ammonium exposure during the cleavage stage [7, 14]. Glutamine metabolism plays a central role in nitrogen balance, as glutamine can be converted to glutamic acid and subsequently to α-ketoglutarate, a key intermediate in multiple metabolic pathways. These include ammonia detoxification via the urea cycle, amino acid biosynthesis, and the synthesis of nitrogenous bases required for nucleic acid formation. Despite extensive research, the mechanisms by which excessive protein metabolism negatively affects reproduction and fertility remain incompletely understood. Notably, no studies to date have reported ammonia synthesis or concentration changes across different embryonic developmental stages in Wistar-Imamichi rats *in vivo*. Therefore, the aim of the present study was to evaluate ammonia concentrations in embryonic fluid, maternal serum, placental tissue, and kidney tissues of both dams and offspring during different stages of embryonic development in Wistar-Imamichi rats.

II. Materials And Methods

Animal Preparation

Two to three month old Wistar-Imamichi rats weighing 220–260g were used in this study. Animals were maintained under controlled environmental conditions at a room temperature of 21±1°C with a 12 h light/12 h dark cycle (lights on at 06:00 h). Rats were provided ad libitum access to a commercial pellet diet (Labo MR Breeders; Nihon Nosan Kogyo Co., Ltd., Yokohama, Japan) and tap water throughout the experimental period. The reproductive status of female rats was monitored daily by vaginal smear examination. Females identified in proestrus were cohabited overnight with fertile males. The presence of a vaginal plug and/or spermatozoa in vaginal smears the following morning was considered evidence of successful mating, and this day was designated as day 1 of pregnancy. Pregnant rats were euthanized by cervical dislocation between days 10 and 20 of gestation. Embryonic fluid, maternal kidney, fetal (kit) kidney, placenta, and blood samples were collected immediately. All samples were weighed and stored at −20°C until further analysis.

Sample Preparation

Embryonic fluid, maternal kidney, fetal kidney, and placental tissues were processed by centrifugation at 2000×g for 5 min to remove tissue debris. The resulting supernatants were collected and used as embryonic fluid, maternal kidney fluid, fetal kidney fluid, and placental fluid for subsequent analyses. Blood samples were collected into capped disposable plastic tubes and centrifuged at 2000×g for 10 min to obtain serum. The supernatants from tissue samples and serum were analyzed immediately for ammonia concentrations.

Ammonia Determination

Ammonia concentration was determined using a colorimetric indophenol reaction based on a two-step process. Briefly, 10 µL of each sample (embryonic fluid, maternal kidney fluid, fetal kidney fluid, and placental fluid) or 20 µL of serum was mixed with 2 mL of sodium pentacyanonitrosylferrate (III) dihydrate solution (Nacalai Tesque Inc., Kyoto, Japan). Subsequently, 2 mL of sodium hypochlorite solution (Wako Pure Chemical Industries Ltd., Osaka, Japan; and Nacalai Tesque Inc., Kyoto, Japan) was added, and the final volume was adjusted to 5 mL with distilled water.

The mixture was vortexed thoroughly and incubated at room temperature for approximately 30 min until maximum blue color development occurred. Absorbance was measured at 625 nm using a spectrophotometer (Model DU-640; Beckman Instruments Inc., Fullerton, CA, USA). All experiments were performed in five replicates. Ammonia concentrations were calculated from a standard curve prepared using ammonia standards ranging from 0 to 0.02×10^{3} µg/mL. Values were converted to micromolar concentrations using the conversion coefficient $A = 1000\times (17.03\ g\ mol^{1})$. Standard curves were generated by linear regression analysis, and the mean coefficient of determination from five curves was $R^2 = 0.9997$ ($Y = 4.95x \pm 0.0005$).

Statistical Analysis

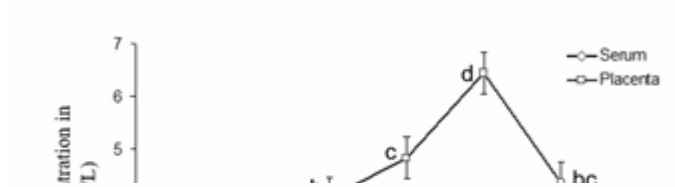
All data are expressed as mean±standard error of the mean (SEM). Statistical analyses were performed using Fisher's protected least significant difference (FPLSD) test. The NCSS (Number Cruncher Statistical

System) software, version 5.01, was used for all analyses. Differences were considered statistically significant at $P < 0.05$.

III. **Results**

Ammonia Concentrations in Placenta and Blood Serum

Ammonia concentrations in the placenta and maternal blood serum at different embryonic developmental stages (days 10–20 of gestation) are presented in Fig. 1. Ammonia levels in both placenta and blood serum increased significantly ($P<0.05$) from day 10 to day 16 of gestation. Thereafter, ammonia concentrations decreased significantly ($P<0.05$) at days 18 and 20. The mean ammonia concentrations in the placenta were 123±1.0, 385±2.0, 407±1.0, 482±1.0, 644±2.0, and 435± 0.6 µM at days 10, 12, 14, 16, 18, and 20, respectively. Corresponding ammonia concentrations in blood serum were 205±2.0, 232±0.2, 313±0.5, 367±0.3, 650±0.2, and 180±0.2 µM, respectively. Ammonia concentrations in both placenta and blood serum were significantly higher ($P<0.001$) at day 16 compared with all other developmental stages.



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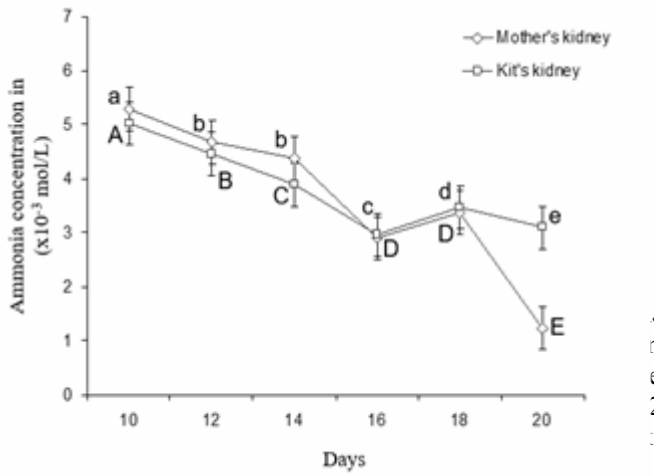

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Values are expressed as mean ± SEM of five

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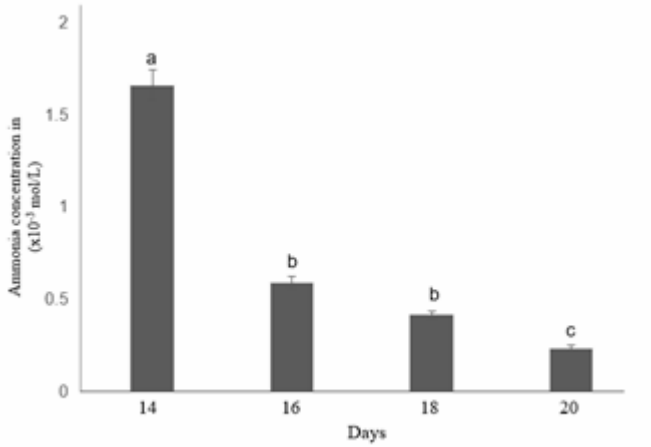

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concentrations in embryonic fluid, maternal
bryonic developmental stages (days 10–20 of gestation) in Wistar-Imamichi rats. Our results demonstrate clear developmental stage dependent changes in ammonia concentrations across these tissues. Notably, ammonia concentrations peaked at day 16 of gestation in the placenta and maternal blood serum, followed by a significant decline at later stages. The mechanisms underlying ammonia accumulation and its variation during development have been previously described in ovarian follicles and other reproductive tissues [2]. Higher ammonia concentrations during early and mid-embryonic development may reflect increased metabolic activity associated with rapid cell proliferation and differentiation. In addition, elevated ammonia levels may be related to higher protein content and enhanced

amino acid turnover during early developmental stages, as reported in earlier studies [3, 5]. As development progresses, the observed decline in ammonia concentrations may result from improved metabolic regulation, increased tissue differentiation, and enhanced nitrogen disposal mechanisms. Previous studies have suggested that lower ammonia concentrations in larger follicles may be due to dilution effects associated with rapid fluid accumulation [14, 10]. In addition, ammonia uptake by surrounding cells may be influenced by ionic composition, particularly potassium and chloride concentrations, which can inhibit ammonium transport through competitive interactions with transport proteins [5]. Although follicular dynamics were not directly assessed in the present study, similar regulatory mechanisms may operate during embryonic and placental development *in vivo*.

The elevated ammonia concentrations observed during mid-gestation are of particular interest, as numerous studies have demonstrated that ammonium exposure can adversely affect embryonic and fetal development. Experimental studies using animal models have shown that ammonium concentrations ranging from 18.8 to 300 μM impair blastocyst development, reduce inner cell mass (ICM) cell numbers, increase apoptosis, and negatively affect implantation and fetal growth [7,15,16]. Moreover, prolonged exposure to ammonium during early embryonic stages has been associated with increased fetal abnormalities and reduced viability [7, 15, 16]. The ammonia concentrations observed in the present *in vivo* study fall within ranges previously reported to exert biological effects, suggesting potential physiological relevance. Embryonic sensitivity to ammonium appears to be developmentally regulated. Prior to compaction, embryos possess a limited capacity to regulate intracellular pH, metabolism, and cellular homeostasis. Following compaction, embryos acquire enhanced adaptive mechanisms that improve tolerance to metabolic and environmental stressors. Edwards *et al.* [16] demonstrated that embryos gain an increased ability to regulate intracellular pH after compaction, while similar improvements have been observed for resistance to osmotic and metabolic stress [17]. These developmental transitions may explain the declining ammonia concentrations observed at later gestational stages in the present study. Ammonium exposure has also been shown to alter gene expression in blastocysts, including suppression of genes involved in metabolism and development, as well as disruption of imprinting related genes such as H19 [3, 15]. In particular, ammonium reduces expression of the glucose transporter Slc2a3, which mediates glucose uptake from the maternal environment, thereby impairing glucose metabolism and ATP production [15, 18, 19]. Reduced glucose availability can compromise embryonic viability and subsequent fetal development. Importantly, some of these gene expression changes may originate during early embryonic stages but only manifest phenotypically later in development, highlighting the long-term consequences of early metabolic stress. The precise mechanisms by which ammonium disrupts embryonic development remain incompletely understood. One proposed mechanism involves alterations in intracellular pH. Exposure of early embryos to ammonium has been shown to decrease intracellular pH [15], and pH disturbances are known to interfere with mitochondrial distribution, ATP delivery, and transcriptional activity [20–23]. Alternatively, ammonium may directly impair energy metabolism by inhibiting mitochondrial shuttle activity or glycolytic enzymes, as demonstrated in other cell types [24–27]. Whether similar mechanisms operate in embryonic tissues *in vivo* requires further investigation. Numerous studies in animals and humans have reported negative effects of ammonium on embryo development, pregnancy outcomes, and fetal growth [2, 3, 10, 5, 7, 8, 15]. While many of these studies employed *in vitro* culture systems with exogenously added ammonium, they provide important mechanistic insight into the potential biological impact of ammonia observed *in vivo* in the present study.

V. Conclusion

This study demonstrates that ammonia concentrations in embryonic fluid, placenta, kidneys, and blood serum vary significantly across embryonic developmental stages in Wistar-Imamichi rats, with peak levels occurring during mid-gestation. These findings suggest that ammonia metabolism is developmentally regulated and may play an important role in embryonic and fetal physiology. Further studies are required to elucidate the regulatory mechanisms controlling ammonia homeostasis *in vivo* and to determine its precise contribution to normal and abnormal embryonic development.

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