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Review Article

Relook and Understanding the Fetal Immune system and Death

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Abstract

The developing fetal immune system can be broadly classified into two phases, i) pre-immune phase when the gestation phase of the fetus is equal to or less than 16 weeks and the ii) Hypo-immune phase, when the gestation is 17 weeks or older. Between 1977 and 2002, Bhattacharya et al., showed that the fetus post 16 to 17 weeks of gestational age has the ability to respond weakly to antigenic stimulation at low doses, and an increased response with high immune stimulation, at larger immunogen doses or in the presence of a strong stimulant. The response of the pre and hypo-immune fetal system with respect to intra-amniotic and intra-fetal toxoid administration was demonstrated in the form of various antigens like tetanus toxoid (dose range 0.5 cc to 4 cc) alone or in combination with levamisole, cimetidine, 20% Bovine Serum Antigen alone, double antigens, maternal buffy coat alone, 5 cc of amniotic fluid alone, 1 cc of Glutamate BCG alone and 2 cc of normal saline alone. However experiments with BCG could not be investigated further due to ethical restrictions. It was observed that with increase in gestational period, the rate of abortion decreased including the cumulative abortion failure rate indicating the maturation and development of the fetal immune system. This obvious and expected antigenic versus host challenge, interaction, stimulation and varying degree of response with respect to different antigens was shown by Bhattacharya et al., clinically for the first time which can help in developing a new strategy or concept for intra-fetal or intra-amniotic immunization post 20 weeks of gestation.

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Introduction

Edward Jenner is credited for the development of the first vaccine for smallpox in 1789. He also coined the term vaccine which came from the word variolae vaccinae [1]. Louis Pasteur during the latter half of the 19th century included the term vaccine for all new protective inoculations [2]. Vaccines can be therapeutic and prophylactic in nature [3,4]. The process by which vaccines are administered is called vaccination and it is by far the most effective way of preventing infectious diseases against hepatitis & Polioviruses, measles and tetanus to name a few.

Vaccine Production Technology

Poliovirus is one of the best examples of modern day vaccine and vaccination. In Poliovirus type of vaccination, two types of vaccines are used i) Chemically or formalin induced inactivated (injection) and ii) Heat attenuated which is used orally and the most preferred choice. These vaccines are produced after culturing them on *Rhesus* monkey kidney cells [5].

In 1960, there were widespread contamination reports that these *Rhesus* monkey kidney cells used for preparing the Poliovirus vaccines were contaminated with monkey simian induced virus or *SV40*. These *SV40* were found to be in the oral vaccines and is also the cause of tumors in rodents and cancer in humans [6]. *SV40* is not pathogenic in monkeys but has been found to be pathogenic in humans. There are some claims that this transmission of *SV40* from monkeys to humans via contaminated kidney cell cultures used for the production of polio vaccination is now the cause of HIV which has been refuted [7].

The modern day production of vaccines can be in four simple steps. i) Generation of the antigen where recombinant DNA technology is used. Here, the DNA is grown and harvested from chicken embryos or fertilized eggs in bioreactors. These recombinant proteins are then generated via cell culture techniques. ii) The second step involves the isolation and the separation of the antigen from the cells and proteins [8]. iii) The third step is the process of antigenic protein purification for producing a high quality product. vi) The final step is the addition of an adjuvant. The role of the adjuvant is to enhance the recipient's immune response. By adding a stabilizer, the vaccine is then formulated and then packed in a vial or a syringe with sterile stoppers in following GMP to avoid contamination [8].

Pre-Term Birth and Immunity

Pre-term birth or PTB is a common problem in both developed and developing countries. PTB can be defined as the birth occurring prior to 37 weeks of gestation [9]. PTB is further classified into three groups, i) late or 32-36 completed weeks of gestation, ii) early or less than 32 completed weeks of gestation and iii) the very early which is less than 28 weeks of gestation [10,11]. It accounts for approximately 13% of all births in the United States and 8% in Australia [12,13]. In 2015 among the 5.9 million deaths under the age of 5 years, 1.055 million babies died due to pre-term complications which was incidentally the leading cause for infant mortality [14]. It is now possible to have a 90% survival chance of an infant born even at 27 weeks of

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• Page 2 of 7 •

gestation and weighing less than or equal to 900 gm [15]. However, long term survival tendencies of infants born before 27 weeks have shown to be extremely poor due to chronic lung diseases, premature retinopathy and intra cranial hemorrhage [15-17].

The pathophysiology of pre-term birth is very complex and is often multifactorial in nature. It is a highly complex pathological disease which is still not properly understood [18]. The etiology of pre-term birth included diabetes, hypertension, addiction, obese or underweight, cervical disease, utero-placental ischemia, stress, infection and inflammation of the mother [19]. PTB death can be also due to deplorable socio-economic conditions, immature immunity and susceptibility to a broad spectrum of infections both in and ex-utero due to under developed innate and adaptive immune system [20]. Intra-uterine inflammation is also responsible for low production of cytokines in the preterm infants. Cytokines and chemokines play a major role through antigenic presentation and activating the B and T cells [21]. Through the secretion of cytokines and chemokines and presentation of antigen, monocytes/macrophages can activate other immune cells such as B cells and T cells [22]. The adaptive immunity matures after term birth which progressively develops with age. However, in cases of preterm born infants, the monocytes have reduced cytokine production, with equivalent efficiency in phagocytosis and intracellular pathogen disruption [21] (Tables 1 and 2).

Year	Weight of the Infants
1940 to 1950	Infants weighing less than 1 kg considered as still born [23].
1950's	Development of the apgar score (Appearance, Pulse, Grimace, Activity, Respiration) [23].
1960' s	1 kg infant started to survive and use of ventilation [23].
1970's	Limit of viability or the minimum age a baby can survive ex utero like less than 24 weeks [23].
1980's	Infants of 24 weeks and birth weight of 500-600 gram were rare [23].
1990's	Infants of 24 weeks and birth weight of 500-600 gram were common. Introduction of surfactants/antenatal steroids [23].
2000's	Increase in incidence of successful preterm births [23].

Table 1: The following table describes the obstetrics and pediatric management of preterm birth in the last 60 years in the US [23].

Appropriate Gestational Age (APA in weeks)	Weight	Category
23	45-550 grams	Babies under 1500 grams are considered as very low birth weight or VLBW and babies less than 1000 grams are often defined as Extremely Low Birth Weight (ELBW) [23].
24	500-650 grams	
25	600-750 grams	
26	700-850 grams	
27	850-950 grams	

Table 2: Correlation of birth weight with gestational age [24].

Intra-uterine infection of the mother has been observed to be associated with chorioamniotis and funistis [24,25]. Gomez et al., have demonstrated that presence of more than 11 pg of fetal cord blood plasma IL-6 concentration is frequently associated with microbial infection and invasion of the amniotic cavity which the infant's immune system is unable to cope with [26]. This elevated level of cord blood IL-6 has been predicted to be the cause of broncho-pulmonary dysplasia along with many inflammatory lesions in the chorio-amnion [27,28]. Oligohydramnios is also found to be associated with elevated fetal cord blood IL-6 thereby causing a range of neonatal morbidities [29].

Duggan et al., on the other hand has shown that during the time of intra-uterine infection, the fetal lymphocytes are activated thereby elucidating a fetal adaptive immune response [30]. Further, there is evidence suggesting that intra-uterine infection is linked to fetal inflammation. This intra-uterine inflammation increases the risk of early sepsis and the fetus develops the ability to respond such infections at the later gestation stages [31].

From the below data, our group observed that with the increase in the gestational week (16 to 20 weeks and above) the success rate of abortion declined. In the 16-19 weeks or more gestational period, there was a gradual increase in the induction-abortion interval. The success rate (if there is a cut off period of one month) of a single intra amniotic injection of 2 cc schedule progressively comes down from 94.74% in the 8-11 weeks group to 88.61 % in the 16-19 weeks group and eventually to 73.67% in the 20 weeks or more group [32] (Table 3).

Disscussion of the reaction on the Fetal Immune System from 8 to 20 Weeks of Gestation (Table 4)

Previous investigations reported that the developing fetus is capable of mounting a highly efficient immune response with respect to congenital infections [33-38]. Bhattacharya et al., and his groups for the first time demonstrated the response of the pre and hypo-immune fetal system with respect to intra-amniotic and intra-fetal toxoid administration on 1056 mothers. In normal congenital infections, the blood placental and the fetal membrane barriers are all destroyed resulting in destruction of the fetus. However, in this case due to toxin administration, the fetus is destroyed initially and then followed by the placenta. In the first case it is the maternal infection which induces destructive changes to the fetomaternal and the blood placental barrier.

From the above data, Bhattacharya and his colleagues between 1978-2002, showed unknown fetal reactions with respect to varying doses of tetanus toxoid from 1 to 4 cc with single intra-amniotic injection, 2 cc of 20% Bovine Serum Albumin (BSA), 10 cc of maternal whole blood from leukocytes or the buffy coat, 5 cc allogeneic amniotic fluid from different mothers, bacterial antigen like sterodin, double antigen & glutamate BCG which showed the maximum potential antigenic reaction up to 15 gestation weeks [33]. Further the data by Bhattacharya et al. between 1978-2002, showed that there is a massive hemorrhage along with deformity in the fetal structures and congestion of the viscera gestational in fetuses with gestational age between 9 weeks to 16 weeks [33]. Tetanus toxoid application showed mononuclear cell infiltration, thrombosis, and hemorrhage in most of the fetal organs including the placenta leading to abortion thereby implying that even in a sterile environment like the amniotic fluid of the fetus can respond to antigenic challenge which are dependent on a number of factors like type/route/virulence and dosage of the antigenic assault [33].

The aforementioned might explain the presence of *Treponema* which at a higher concentration can abruptly trigger death and deformity of the fetus depending upon its gestational stage resulting in either a hyper acute reaction (upto 16 weeks) or a chronic above 17 weeks [33]. A normal immune system follows a very delicate and highly coordinate immune axis which in case of exaggerated responses results in hypersensitivity reaction which may be of different types for example Type I, II, III, IV & V [33]. Antigen elimination via humoral and cellular processes is linked to inflammation and its mediators and has a positive role in promoting efficient elimination

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• Page 3 of 7 •

of the foreign pathogen. When the embryo is developing in the hypo-immune stage all the process of inflammation and its responses are actually in the developing stage resulting in a poor co-ordination of the fetal immune system [33]. This poor coordination can have a direct negative effect on the growing fetal system thereby causing dysregulation and inflammation leading to tissue damage and death of the fetus. 17 weeks onward, the fetal immune system starts to become more matured than what it was previously [33].

No. of cases	Weeks of Gestation	Type of Antigen Used	Success Rate of Abortion (in %)	Condition of Abortus (living)
19	8 to 11 weeks	Tetanus toxoid 2 cc single intra-amniotic injection schedule [32]	94.74	None
319	12 to 15 weeks	Tetanus toxoid 2 cc single intra-amniotic injection schedule [32]	94.05	None
316	16 to 19 weeks	Tetanus toxoid 2 cc single intra-amniotic injection schedule [32]	88.61	36 fetuses all above 16 weeks
34	20 weeks and above	Tetanus toxoid 2 cc single intra-amniotic injection schedule [32]	73.67	All fetuses living

Table 3: Experiment and analysis of intra-amniotic instillation of toxoid in fetuses between 8-19 weeks of gestation and their abortion responses [32].

S. No	No. of Cases	Weeks of Gestation Range	Antigen Used (Dose)	Comments Cut off Period 14th day
1	20	9-19 weeks. Mean gestation with SD 13.8 +/- 1.2	½ cc tetanus toxoid (Single injection) [32].	12 patients did not abort on the 7th day, 6 patients did not abort on the 14th day [32].
2	14	8-19 weeks. Mean with SD 14.4 +/-2.4	1 cc tetanus toxoid (single injection) [32].	10 patients did not abort on the 7th day, 4 patients did not abort on the 14th day [32].
3	16	9-19 weeks. Mean with SD 12.6 +/-1.2	1.5 cc tetanus toxoid (single injection) [32].	6 patients did not abort on the 7th day, 4 patients did not abort on the 14th day [32].
4	10	8-18 weeks. Mean with SD 14.6 +/-2.8 weeks.	3 cc tetanus toxoid (single) injection intra-amniotically [32].	6 patients did not abort on the 7th day, 3 patients did not abort on the 14th day [32].
5	12	8-19 weeks. Mean with SD 14.6 +/-2.4	4 cc single injection intra-amniotically [32].	8 patients did not abort on the 7th day, 4 patients did not abort on the 14th day [32].
6	12	12-18 weeks. Mean gestation with SD 12.6 +/- 1.4	Single intra fetal injection of 2 cc under USG`[32].	6 patients did not abort on the 7th day, 2 patients did not abort on the 14th day [32].
7	10	8-18 weeks. Mean with SD 14.6 +/-2.4	Single intra amniotic injection of 2 cc tetanus toxoid and 300000 IU of Vitamin A [32].	4 patients did not abort on the 7th day, 7 patients did not abort on the 14th day [32].
8	12	8-18 weeks. Mean with SD 13.8+/-0.8	Tetanus toxoid 2 cc with levamisole orally to mother [32].	7 patients did not abort on 7th day. 3 patients did not abort on the 14th day [32].
9	16	8-19 weeks. Meanwith SD 14.2+/- 1.4 weeks	2 ml of single fixed dose of intra-amniotic injection [32].	6 patients did not abort on 7th day. 4 patients did not abort within 14th day [32].
10	14	8-19 weeks. Mean gestation with SD 13.6+/- 1.4 weeks	2 cc of intra amniotic tetanus toxoid and oral cimetidine 400 mg BID to mother [32].	8 patients did not abort on 7th day. 6 patients did not abort within 14th day [32].
11	26	11-15 weeks. Mean gestation with SD 13.2+/- 3 weeks	1 cc of Glutamate BCG [32].	Mothers who were Mantoux test negative were enrolled. Mantoux test positive and ELISA Tb IgA, IgG and IgM (+) cases were discarded from study design [32].
12	14	10 to 16 weeks. Mean gestation with SD 13.4 +/- 1.4	0.5 cc of dyphteria and 0.5 cc antigen commonly known as double antigen [32].	6 patients aborted within 7 days. 10 patients cumulatively aborted within 14 days [32].
13	14	11 to 18 weeks. Mean gestation with SD 14.6 +/- 1.2	2 cc 20% BSA antigen [32].	8 patients did not abort on the 7th day. 4 patients did not abort on the 14th day [32].
14	10	8 to 14 weeks. Mean gestation with SD 13.4 +/- 1.2	10 cc of maternal whole blood [32].	6 patients did not abort on the 7th day. 3 patients did not abort on the 14th day [32].
15	16	9 to 15 weeks. Mean gestation with SD 12.6 +/- 0.8 weeks	Buffy coat 10 cc of maternal blood [32].	11 patients did not abort on the 7th day. 8 patients did not abort on the 14th day [32].
16	12	12 to 16 weeks. Mean gestation with SD 14.0 +/- 1.8 weeks	5 cc of allogeneic amniotic fluid of the allogeneic mother [32].	5 patients did not abort on the 7th day. 7 patients did abort on the 14th day [32].
17	10	9 to 18 weeks. Mean gestation with SD 13.6 +/- 2.4 weeks	Bacterial antigen (polysaccharide 2cc) [32].	6 patients did not abort on the 7th day. 4 patients did abort on the 14th day [32].
18	106	10 to 20 weeks. Mean gestation with SD 14.8 +/- 1.8 weeks	2 cc of normal saline with thiomersol and aluminium phosphate [32].	All 4 patients had hype pyrexia and incomplete abortion. Amniotic fluid bacterial culture suggested haemolytic streptococcus and staph. Aureus in 3 cases, cervical culture positive for *E. Coli* in one case [32].
19	15	13-16 weeks. Mean 13.6 +/- 1.4 weeks	2 cc of single fixed intra-amniotic injection [32].	6 patients did not abort within 7 days and 2 patients did not abort within 14 days [32].

Table 4: Reaction on the fetal immune system from 8 to 20 weeks of gestation.

Citation: Bhattacharya N, Sengupta P (2018) Relook and Understanding the Fetal Immune system and Death. J Vaccines Res Vaccin 4: 007.

• Page 4 of 7 •

Serial No. 1: n=20, with 0.5 cc (1/2 cc) the failure rate on the seventh day is 60% and on the 14th day it is 30% cumulative failure. Gestational week of the fetuses were 9-19 weeks.

Serial No. 2: n=14, with 1 cc the failure rate on the seventh day is 71.42% and on the 14th day it is 42.85% cumulative failure. Gestational week of the fetuses were 8-19 weeks.

Serial No. 3: n=16 with 1 ½ cc tetanus toxoid, the failure rate on the seventh day is 37.5 % and on the 14th day it is only 25%. Gestational week of the fetuses were 9-19 weeks.

Table No. 1: 688 patients injected with 2 cc tetanus toxoid the failure on the seventh day is 47.2 and the cumulative failure is 27.6 %. Gestational week of the fetuses were 8-20 weeks.

Serial No. 4: n=10 cases, with 3 cc tetanus toxoid dosage the failure rate is 60% on the seventh day and 30% cumulative failure on the 14th day. Gestational week of the fetuses were 8-19 weeks.

Serial No. 5: n= 12, with 4 cc tetanus toxoid dosage the failure rate is 60 percent on the 7th day is 66.6% and on the fourth day it is only 33.3%. Gestational week of the fetuses were 8-19 weeks.

Serial No. 6: n=12 patients, gestation week: 12-18 weeks, Single intra fetal injection of 2 cc under USG guidance delivered locally, rate of abortion on the 7th day is 50% and perhaps very high on the 14th day which was 83.33%.

Serial No. 7: In the presence of a fetotoxic or immunomodulatory substance like Vitamin A (dosage of 300000 IU) injected with tetanus toxoid there was a failure of 70% cases on the 7th day and a cumulative 40% abortion failure was noted on the fourteenth day.

Serial No. 8: 58.33% cases failed to abort on the seventh day and 25% cases cumulatively failed to abort on the fourteenth day when oral levamisole (150 mg OD × 7 days) was utilized along with 2 cc of Tetanus toxoid in order to understand whether maternal T cell stimulation or modulation of T cell function pharmacologically can make the induction of abortion interval more predictable.

Serial No. 9: It was found 62.5% cases aborted within seven days and 75% cases of abortion happened within the fourteen days.

Serial No. 10: It was found that 8 cases did not abort on the seventh day (57.14 percent) and 6 cases did not abort on the fourteenth day (42.85% cumulative failure).

Serial No. 11: No abortion was observed after injecting the mother with 1 cc of Glutamate BCG. However, progressive diminution of the size of the uterus was observed and on the 14th day of hysterotomy the uterus was found to be practically free of the conceptus. On repeat, 26 such cases with gestation period between 11 to 15 weeks, the result remained the same on the 14th day. Ethical committee did not permit to do the experiments on more than 15 weeks old fetuses.

Serial No. 12: n=14 cases, 42.85 % failure was noted on the 7th day and cumulative 28.51% cases of the failure on the fourteenth day when 1 cc of double antigen having 0.5 cc of diphtheria toxoid along with 0.5 cc of pertussis was injected intra amniotically in fetuses of 10-16 weeks of gestational age.

Serial No. 13: n=14 cases, gestation week=11 weeks to 18 weeks, application of 20 percent BSA 2 cc, intra amniotically, 57.14 % of the cases did not abort on the seventh day and cumulatively 28.57% cases did not abort on the 14th day.

Serial No. 14: n=10 cases, gestational week = 8 to 14 weeks, 10 cc of maternal whole blood was applied and on the 7th day there was abortion in 40% of the cases, while 70% cases aborted on the 14th day thereby raising the question of the critical amount of inadvertent fetomaternal exchange occurring during normal pregnancy including the problems of surgery of the unborn in the early weeks from the point of triggering off of abortion.

Serial No. 15: n=16, gestational age 9 to 15 weeks, 10 cc of freshly collected maternal buffy coat injected revealed abortion in 31.25 % cases on the seventh day and cumulatively 50% within fourteen days.

Serial No. 16: n=12, 12 to 16 weeks, gestational age, 5 cc of single injection of allogeneic amniotic fluid revealed 7 cases or 58.83% did not abort within 14 days cumulatively and 5 cases or 41.66% did abort on the seventh day.

Serial No. 17: n=10, gestational age= 9 to 18 weeks, application of bacterial antigen (polysaccharide 2 cc) revealed 60 percent cases did not abort on the seventh day and 40 percent cumulative failure was noted on the fourteenth day.

Serial No. 18: n=106 cases, gestational age= 10 to 20 weeks, revealed no abortion within 1 month in 102 patients whereas 4 patients were aborted on the 18th hour after injection and 42 hours injection, 116 hours and 22 hours respectively.

Serial No. 19: n=15 cases, gestational age= 13 to 16 weeks, revealed an 86.66 % success rate on the 14th day.

Development of the fetal immune system

The developing fetal immune system is very much unlike an adult immune system which through a series of exposures develops a long lasting immunity that helps in protecting the adult from re-infection [39]. The in-utero fetal environment is like a closed system and the fetus has minimal exposure to infections and pathogens therefore rendering the fetal immune system to be an inexperienced and immature one [39]. Further the series of responses required in immune coordination are immature or lacking and the newborn remains susceptible to infections in the initial postnatal period. However, this does not imply that the fetal immune system is totally inert [39]. At 5 weeks of gestation, the bone marrow and the fetal liver play an active role and Polymorphonuclear (PMN) leucocytes are the first to be identified [40]. Mature PMN's are detectable by 14 weeks of gestation although the concentration of these cells remains low at 2% continuing up to 22-23 weeks of gestation. This is compensated by the high concentration of hematopoietic progenitor cells which shifts its production from the liver to the bone marrow [41]. In the meantime, between 6-8 weeks of gestation period, B and T Lymphocytes and Natural Killer (NK) cells are detectable. Mature T and B cells appear in the 3rd trimester of the fetus and by 13 weeks B cell precursors are found in the bone marrow [39]. Up to 8 to 9 weeks, the fetal thymus still contains a high number of T cell precursors. Monocytes do not appear in the fetal blood circulation before 20 weeks of gestation [39]. At 30 weeks of gestation, the monocyte concentration increases to 3-7%. This is similarly observed in cases of NK cells where the concentration of these cells increase in the fetal circulation with increase in the gestational period thereby explaining the fetal susceptibility to infections in the initial developmental phases [39].

Fetal natural immunity/ humoral component

Complement proteins start to appear between 8 to 14 weeks of gestation and opsonins remain reduced in the new born due to the deficiency of the complement/IgG and IgM antibodies [42]. During 6 to 14 weeks of gestation, very low concentration of complement components can be found. Fibronectin is another component of the natural immunity which is essential for cellular migration, proliferation, differentiation, wound healing and for embryo development. Fibronectin has been shown to be down regulated in cases like sepsis, Intra-Uterine Growth Retardation (IUGR), respiratory distress syndromes and fibrosis resulting in organ dysfunction [43]. Down-regulation of fibronectin occurs at the neonatal stage although [44]. C-Reactive

Protein (CRP) is also synthesized in the fetus as it helps in the opsonisation process and rapid clearance of bacteria. Lactoferrinis another important component of the natural immunity of the fetus that helps in endothelial adhesion and aggregation and is found to be in low concentrations in the neonatal cord blood [45].

T cell ontogeny of the fetal immune system

During the 6th and 7th week of gestation the thymus develops as an outgrowth of the pharyngeal pouches and by the 10th week of gestation the cortex and the medulla region appears to be distinct [46]. Lymphocytes start to appear between the 9th and 10th week along with the epithelial cells [47]. The Hassall's corpuscles appear by the 1st trimester. In the thymus, maturation of the T cells occurs along with the CD1, CD2, CD5, CD7 and CD3. With increase in the gestational period the mature CD4 and CD8 cells leave the thymus and migrate to the secondary lymphoid tissues [48]. Cells lacking both the CD4 and CD8 cells are termed as stem cells which have the capacity to turn into CD4 or CD8 cells due to the expression of CD25 receptor which has a high affinity towards the production of IL-2 responsible for a high CD4 and CD8 turnover. The number of CD3 positive cells which appears in the fetal liver and spleen by the end of second trimester and expressing both CD4 and CD8 cells also increases with the increase in the gestation period and produces more than half of the T lymphocytes by the end of 22nd week of gestation. CD4 and CD8 ratio in the cord blood is normally less than the adult ratio of 2:1 [37].

Role and production of lymphokines

By 12th week of gestation period, lymphocytes from the thymus can respond to foreign antigens [49]. Production of some lymphokines as circulatory cells get diminished including memory T cells in the neonate stage and the fetal cells can start to exhibit normal antigen specific cytotoxicity.

B lymphocytes and antibody production in the fetus

B cells develop in two stages. The first stage is through undifferentiated stem cells which is an antigen independent process in the fetal liver and bone marrow. In the second the cells undergo lymphoid differentiation which is the antigen dependent process [50]. The pre B cell where clonal diversity arises appears first during the 7th to 8th week of gestation in the fetal liver with further expansion in the bone marrow with the increase in gestational period also gives rise to the immature B cells in the fetal liver at 8-9 weeks of gestation. It also expresses the complement receptors and IgM [51]. By the 12th week of gestation, the fetus has high levels of B circulating lymphocytes greater or less equal to the adult. Also, by the 12th week the appearance of IgA and IgG can be observed along with IgM in the fetus by 8th week of gestation and the concentration of IgM increases from 6 mg/dl at less than 28 weeks to 11 mg/dl at term which is approximately 8% of the maternal level [52]. IgG remains high at birth and is mostly passed through the placenta and reaches adult levels 4-6 years of post-natal period and IgA appears at the 30th week of gestation and reaches adult levels at puberty. In the initial phase of development of the fetus, due to the presence of a sterile environment in the uterus, the antibodies remain inert in their function until the time of birth [45].

Prospect of vaccinating the fetus

The concept of vaccine strategy in young children is to develop a strong immune response in around 2-4 months. Antibodies normally appear in the 6th-7th month thereby failing to prevent infections that the new born may be exposed to in the first month of birth [53]. There can be several strategies in place like maternal immunization, neonatal immunization/ A third and new prospect of immunization can be the intra-amniotic or intra fetal injection post 20 weeks of gestation [54,55]. Although in theory, the third strategy might prove to be an attractive proposition for providing immunity to the fetus from the preterm developmental stage itself, practically from our previous research experience and datas, it can be concluded that such interventions and strategies may be devised to the fetus only after 20 weeks of gestation. Bhattacharya et al., between 1978 to 2002, reported that there is abortion but no fetal death post 18 weeks in the growing fetus and strongly rejects the vaccination of the unborn via the intra-amniotic and intra-fetal route because of its potential negative impact on the growing fetus as observed through histology studies including poor antibody response before 20 weeks of gestation [56]. Our research further indicated that above 17 weeks of gestation, the degree of dissolution of the fetus also dramatically decreases, the highest being at 20 or above 20 weeks of gestation which might be either due to the appearance of the HLA system or maturation of the immune system. However, the above findings need more detailed investigations [57].

Lack of antibody formation and reduction in the immune and neuro co-ordination in the developing fetus can often be lethal if they are tested with antigenic stimulus. Through our experiments we have observed increase in the fetal liver weight, thymus and spleenic weight after tetanus toxoid stimulation including elevated essential hepatic enzymes thereby representing the phenomenon of Graft Versus Host Disease (GVHD) [58]. This is due to the disruption of the thymus schooling of the fetal T-lymphocytes. The pre-immune stage or below 16 weeks of gestation, destruction of the fetus might be inflammation mediated or even triggering of the auto-immune phenomenon due to the presence of a pre-matured participation of the immune system. Experimentation with tetanus toxoid and the delayed response of a tiny (8-150 g) human fetus to its antigenic stimulation in large doses has shown wide variations in the induction-abortion interval. It might be attributed to the lower expressions of the receptors, hypo-immune condition or even neo immature antigens which confers resistance to antigenic stimulation. This hypo-immune response of the fetus can be utilized in fetal cell transplantation as it might not be rejected by the host's body due to failure of GVHD [58-61]. Whether this hypo-immune condition can be of any advantage to develop a fetal immunization strategy still needs a lot of investigations.

Conclusion

On the basis of the above data from the experiments conducted since 1977 till 2002 by Bhattacharya et al., on the developing fetus it can be concluded that the pre mature fetus can mildly respond to antigenic stimulus and might require a larger dose of antigenic challenge for higher stimulation due to its hypo-antigenicity (0.5 cc to 4 cc). This is an important finding implicating the tolerogenic capacity of the fetus at varying degrees of development. This observation is also important in determining the effective dose for vaccination for implementing newer methods of intra-fetal or intra-amniotic immunization against particular infections. It was demonstrated practically for the first time that, in the initial phases of development i.e. during the 8 weeks to 12 weeks of gestation a high rate of fetal loss is evident when challenged with varying dose of antigen thereby confirming the

fact that the immune system remains in a pre-immune phase immune. With increase in gestational period, including the toxoid dose the fetus can perhaps mount a defensive system thereby reducing the rate of abortion and providing a direct evidence of the progressive development and maturation of the fetal immune system. This method can also be an alternative for a clean, safe and cheap abortifacient without any prevailing long term or short term complications observed in poor and developing countries. Further through our experiments we believe that intra-amniotic or intra-fetal injection to the fetus can be detrimental and result in the abortion if given below 20 weeks of gestational development. The concept of fetal immunization in the womb itself post 20 weeks of gestation, can be a novel strategy as it can help the fetus to develop an effective and long lasting immunity with less susceptibility to term and post natal infections. However, the practical challenges in doing so are immense in terms of its procedure and ethics.

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