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MATRIX INTERFERENCE IN DIRECT TOTAL TESTOSTERONE ENZYME IMMUNOASSAY AND ITS ELIMINATION WITH THE USE OF NON-CROSS REACTIVE STEROIDS IN SERUM BASED STANDARDS

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ABSTRACT

While devising a total serum testosterone enzyme linked immunosorbent assay (ELISA) using charcoal stripped pooled serum for making standards, a significant measurement bias caused by a matrix interference was observed when the immobilized testosterone antibody had a relatively high binding for testosterone-horse radish peroxide (testosterone-HRP) tracer. Therefore, to improve the accuracy of the direct total testosterone ELISA, in the present study, pooled serum was first stripped with charcoal and then spiked by non-cross reactive steroids at their higher normal concentration present in the subjects (male and female). Standards prepared in this pooled serum expressed a reduced binding for testosterone -HRP tracer. The results indicate that the matrix interference was removed and the assay performance was improved as compared to standard prepared in the non-spiked charcoal stripped pool serum. This may be because of the presence of non-cross reactive steroids in the serum based standard makes available testosterone free to compete with testosterone - HRP tracer for binding to immobilized testosterone antibody by inhibiting the binding of testosterone to sex hormone binding globulin (SHBG). The direct total testosterone ELISA, developed in the present study, based on non-cross reactive steroid spiked serum standards is accurate, precise over the non spiked serum standards and maintained the merits of direct measurement.

Key-words: Immunoassay, Matrix interference, Direct ELISA.

The competition between plasma proteins and specific antibodies for the analyte is a well documented phenomenon in the area of steroid immunoassay[1,2].

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In direct assays, steroid must be quantitatively displaced from binding proteins. Major steroids (e.g. cortisol/progesterone) bound in serum to corticosteroid binding globulin (CBG) may be displaced by protein binding agents such as 8 - anilino-1-naphthalene sulphonic acid (8-ANS) and salicylate, by proteolytic enzyme, by low pH or by heat treatment[3]. In addition, danazol, dexamethazone and cortisol, which have affinities for CBG, have been used in direct assay for progesterone[3,4]. A disadvantage of some of these displacing agents is that they partially reduce the specific binding of the antibody at their effective blocking concentration[2]. In some cases, these displacing agents also cross-react with the specific antiserum. In addition, the conditions employed for displacing steroid bound to SHBG in direct assays for testosterone and estradiol also place great demand on specificity of the antiserum. These exogenous steroids are potential sources of cross-reaction[5,6]. The complex mixtures of displacing agents put the direct immunoassay at a distinct disadvantage and the performance in terms of sensitivity may be affected significantly[4]. While developing ELISA for testosterone, a significant measurement bias was noticed. Therefore, in the present study, author has successfully developed a direct total testosterone ELISA based on non-crossed reactive steroid spiked serum to eliminate measurement bias and to improve the accuracy of the assay.

MATERIALS AND METHOD

Testosterone, testosterone-3-O-carboxymethyloxime (testosterone-3-O-CMO), adipic acid dihydrazide (ADH), bovine serum albumin (BSA), 1 ethyl-3-(3-dimethyl-amino-propyl)-carbodiimide, HCI (EDAC), N-hydroxysuccinimide (NHS), Freund's complete adjuvant (FCA), gelatin, thimerosal and dextran T-70 were all purchased from Sigma Chemical Company, St. Louis, M.O., U.S.A; Horseradish peroxidase and tetra-methyl-benzidine/H_2O_2 solution was purchased from Pierce Chemical Company, USA and Genei, Banglore, India, respectively. Whereas the microtiter plates were procured from Lab. System, India. All other chemicals and buffer salts were of analytical grade.

Buffers

1. The most frequently used buffer was 10 mM phosphate (10 mM PB), pH 7.0, ($Na_2HPO_4.2H_2O$:0.895 gm/L and $NaH_2PO_4.2H_2O$:0.39 gm/L).
2. HRP conjugate dilution buffer was 10mM acetate (10 mM AB), pH 5.6, CH_3 COONa: 0.84 gm/L and 1 N CH_3COOH 1.5 mL/L), containing 0.1 per cent thimerosal and dextran T-70, 0.3 per cent BSA and 10μg estradiol/mL.
3. Microtiter well blocking and stabilizing buffer was 10 mM PB containing 0.9 per cent NaCl, 0.2 per cent BSA, 0.1 per cent gelatin, thimerosal, dextran T-70, ethylene diamine tetra acetic acid di-potassium salt (EDTA: K salt) and 0.01 per cent gentamicin sulfate.

Antibody Generation

***Primary Antibody*:** Testosterone-3-O-CMO was covalently linked to BSA by activated ester method with modification[7]. In brief, 10 mg of testosterone-3-0-CMO was dissolved in 800 μl of 1:1 mixture of dimethyl formamide and dioxan. Hundred microliters of water containing 20 mg of NHS and 40 mg of EDAC was added to the above solution and the reaction mixture was kept at 4^0C for 24 hours.

The above activated testosterone was added drop-wise to 25 ml of water containing 50 mg of BSA. The reaction mixture was further kept for 24 hours at 4^0C. After 24 hours of incubation, the reaction mixture is extensively dialysed against 10 mM ammonium carbonate buffer. The dialysate is lyophilized and kept at -30^0C for further use for immunization. New Zealand white rabbits were immunized with this conjugate according to the procedure described elsewhere[8].

***Secondary Antibody*:** A group of three goats were immunized with an emulsion of 2.5 ml of FCA in 2.5 μl of saline containing 5 mg of normal rabbit γ - globulin per goat by following the method of Rao et al.[9]. Blood was collected after 12 days of third booster injection and thereafter every 30 days and checked for titer.

Preparation and Dilution of Testosterone-3-O-CMO-ADH-HRP Conjugate

Horseradish peroxidase was activated and coupled with ADH by using periodate method of Wilson and Nakane[10] with some modifications. The testosterone-3-O-CMO was coupled with ADH-HRP by activated ester method with modification[8]. In brief, 10 μl of freshly prepared 0.1M sodium meta periodate solution in water was added to 10 mg of HRP reconstituted in one ml of water and the reaction mixture was kept in dark for 40 minutes. Activated HRP was passed through G-25 column previously equilibrated with 10 mM ammonium carbonate pH 9.3. The brown colour activated HRP was directly collected in a vial containing 100 mg of ADH. Reaction mixture was kept at 4^0C for overnight. After overnight incubation, 10 μl of 5M sodium cyanoborohydride in 1M NaOH was added and reaction mixture was kept at 4^0C for 3 hours. The above reaction mixture was passed through previously equilibrated G-25 column with 10 mM PBS containing 0.1 per cent thimerosal. The brown coloured ADH-HRP conjugate was directly collected and kept at -30^0C for future use.

Five milligram of testosterone-3-O-CMO was dissolved in 400μl of 1:1 mixture of dimethyl formamide and dioxan. 100μl of water containing 10 mg of NHS and 20 mg of EDAC was added to the above solution and the reaction mixture was kept at 4^0C for 24 hours.

One ml of ADH-HRP solution (containing approximately one mg of HRP) was added to the activated testosterone-3-O-CMO and the reaction mixture was

further kept for 24 hours at 4^0C. After incubation, the reaction mixture was passed through G-25 column, previously equilibrated with 10 mM PBS containing 0.1 per cent thimerosal. The brown coloured fractions were pooled in which pinch of sucrose, ammonium sulfate, and BSA were added. The equal volume of ethylene glycol was added to pooled fractions and kept at -30^0C in aliquots for future use.

The optimal dilution of testosterone-3-O-CMO-ADH-HRP conjugate (1:4000) was found out by checkerboard assay. The diluted conjugate was stored in the conjugate dilution buffer at 4^0C for future use. The conjugate solution was stable for more than one year at 2-8^0C.

Preparation of Testosterone Standard in Charcoal Stripped Serum

Deionized water was added to charcoal, stirred for one minute and kept it standing for one hour. Water was decanted and the sides of the beaker were wiped to remove the charcoal. The process was repeated until no more fine charcoal appeared on the surface of the water. The water was filtered by whatman No.1 filter paper. The charcoal was heated overnight in oven at 50^0C and made in powdery form by beating the clumps.

Activated charcoal (50 mg/mL) was added to pooled serum and stirred for two hours at 45^0C. It was then centrifuged at 3000x g for 30 minutes at 4^0C to remove the charcoal. The supernatant was then collected and passed through 0.45μ membrane filter.

Five testosterone-working standards (0.2 ng/mL, 0.6 ng/mL, 2 ng/mL, 6 ng/mL and 20 ng/mL) were prepared in charcoal stripped serum spiked with and without steroids.

Coating of Microtiter Plate

Wells of the microtiter plate were coated with 225μL of diluted testosterone-3-O-CMO antibody by immunobridge technique[11]. 250μL of water diluted normal rabbit serum (NRS; 1:1000) was dispensed in each well of the microtiter plate and incubated for overnight at 4^0C. After incubation, the contents of the plate were decanted and the plate was washed under running tap water. 250μL of diluted second antibody in 10 mM PBS (1:4000) was added to the normal γ-globulin coated wells of the microtiter plate and incubated for two hours at 37^0C. After incubation, contents of the plate were decanted and the plate was washed under running tap water. 225μL of testosterone antibody diluted in 10mM PBS (1:4000) was added to the immunochemically immobilized second antibody through normal γ-globulin coated wells of the microtiter plate and incubated for one hour at 37^0C. At the end of incubation, once again the contents of the plate were decanted and the plate was washed under running tap water. The unoccupied sites of the wells of the microtiter plate were blocked and the wells

were stabilized by adding 250μL of blocking and stabilizing buffer and incubated for one hour at 37^0C. After one hour, the contents were decanted and the plate was dried at room temperature and kept at 4^0C for future use.

Preparation of Substrate Solution

Substrate solution was prepared from TMB/H_2O_2 stock solution. According to the manufacturer protocol, 100μL of TMB/H_2O_2 solution was diluted to 2mL (1:20 dilution) in water. This solution was freshly prepared just before its use.

One Step ELISA Procedure

50μL of testosterone standards or serum sample was added in duplicate to the testosterone-coated wells. 100 μL of testosterone-3-O-CMO-ADH-HRP conjugate was added to all wells and incubated for one hour at 37^0C. After incubation, the contents of the wells were decanted and washed under running tap water for five to six times by filling, decanting and flicking. Finally, for measuring the bound enzyme activity, 100μL of substrate solution was added to all the wells and incubated for 15 minutes at 37^0C. The reaction was then stopped by adding 100μL of 0.5M H_2SO_4 and the color intensity was measured at 450 nm in Tecan-spectra ELISA plate reader.

Blocking of SHBG

Standards were prepared in pooled serum from which endogenous steroids have been stripped off by charcoal. The non-cross reactive C_{18}, C_{19}, C_{21} and C_{27} steroids were added at their higher normal concentrations to subjects (male and female) for blocking the binding of testosterone to SHBG.

Release of Testosterone Bound to SHBG in Unknown Samples

The testosterone-3-O-CMO-ADH-HRP conjugate was diluted in HRP conjugate buffer, which contained 10μg of estradiol/mL. 100μL of diluted conjugate was added to all wells and incubated for one hour at 37^0C. It was observed that the estradiol present in the conjugate buffer bound to SHBG and released the testosterone from it.

Radioimmunoassay (RIA) Procedure

The RIA of testosterone was performed by following the method of Abraham[12].

FINDINGS

Sensitivity

The laboratory tests revealed that the lower detection limit of the assay, i.e., concentration equivalent to Bo-2SD was 0.015 ng/ml of serum after forty-fold determination of Bo binding.

Specificity of Antibody

The testosterone 3-O-CMO-BSA antibody had less than 0.1 per cent cross-reaction with naturally occurring C_{18}, C_{19}, C_{21} and C_{27} steroids except 5α dihydrotestosterone (10%).

Analytical Recovery

The assay ability was tested to quantify serum testosterone accurately. Low, medium and high concentrations (2 to 15ng/mL) of testosterone were added exogenously to three fractions of pooled female serum. After addition, the concentrations of testosterone were determined against standards prepared in with and without steroid spiked charcoal stripped serum and by ELISA and RIA. The results are shown in Table 1, 2 and 3.

TABLE 1

RECOVERIES OF TESTOSTERONE FROM EXOGENOUSLY SPIKED FEMALE POOLED SERUM ASSAYED BY ELISA AGAINST STANDARD PREPARED IN CHARCOAL STRIPPED SERUM WITHOUT STEROIDS

Pooled serum female	Testosterone added (ng/ml)	Expected (ng/ml)	Obtained (ng/ml)	Recovery %
Basal	0		3.0	
Low	2	5	5.1	102
Medium	6	9	9.3	103
High	15	18	18.8	104

TABLE 2

RECOVERIES OF TESTOSTERONE FROM EXOGENOUSLY SPIKED FEMALE POOLED SERUM ASSAYED BY ELISA AGAINST STANDARD PREPARED IN CHARCOAL STRIPPED SERUM SPIKED WITH C_{18}, C_{19}, C_{21} AND C_{27} STEROIDS

Pooled serum female	Testosterone added (ng/ml)	Expected (ng/ml)	Obtained (ng/ml)	Recovery %
Basal	0		1.4	
Low	2	3.4	3.4	100
Medium	6	7.4	7.6	102.7
High	15	16.4	16.0	97.5

TABLE 3

RECOVERIES OF TESTOSTERONE FROM EXOGENOUSLY SPIKED FEMALE POOLED SERUM ASSAYED BY RIA

Pooled serum female	Testosterone added (ng/ml)	Expected (ng/ml)	Obtained (ng/ml)	Recovery %
Basal	0		1.5	
Low	2	3.5	3.4	97
Medium	6	7.5	7.6	101
High	15	16.5	16.8	101

DISCUSSION

It is clear that the ELISA developed by the author for estimation of testosterone in human sample is direct, one step, rapid and simple. In the present method, charcoal stripped pooled human serum (matrix) has been used for the preparation of testosterone standard. The unknown samples or pooled female serum exogenously spiked with low, medium and high concentrations of testosterone estimated against this standard showed a positive significant measurement bias. This may be because of charcoal stripping. The stripped serum is devoid of all the steroids and other low molecular weight compounds. When steroid standard is prepared in this stripped serum, it binds with binding globulin and due to this the amount of free steroid for competition with tracer in matrix remains in a small quantity. Therefore, the inhibition in the binding of tracer is less or in other words, there is a relatively high binding of tracer to immobilized antibody. This in turn causes positive measurement bias. To overcome this hurdle, a non-cross reactive C_{18}, C_{19}, C_{21} and C_{27} naturally occuring steroids at their higher normal concentrations present in male and female was supplemented in the charcoal stripped serum. Due to this supplementation, the binding globulins become saturated and when steroid standard is prepared in this steroid spiked stripped serum, it remains free for competition with tracer in matrix and eliminates the measurement bias.

One of the main problems associated with the direct assay is the matrix interference. Boots et al. [13] estimated that total serum testosterone levels, using commercially available kits, and observed a high degree of variability between-kits which was attributed to level of SHBG. A few of immunoassays other than ELISA have been published for direct estimation of testosterone in serum. These assays employed estradiol, in radioimmunoassay [1] and chemiluminiscence assay [14] as testosterone displacing agent. These displacing agents were supplemented along with label. The estradiol has been used in conjugate buffer as a testosterone-displacing agent from SHBG of unknown serum samples under the ELISA method.

Data presented in Tables 1, 2 and 3 show that the recovery of testosterone from exognously spiked female-pooled serum by ELISA and RIA is ranging from 97.5 to 104 per cent. But the amount of testosterone estimated by ELISA against the testosterone standard prepared in charcoal stripped pool serum, not spiked by naturally occurring C_{18}, C_{19}, C_{21} and C_{27} steroids, shows a positive measurement bias. This is more than 100 per cent at low level and around 22 per cent at higher level, as compared to RIA and ELISA where testosterone standard prepared in steroid spiked charcoal stripped serum. This means that the variations, which exist in the commercial kits, may be because of the use of stripped serum not spiked by naturally occuring steroid for the preparation of standard. In the present study, the non-cross reactive naturally occurring steroid spiked charcoal stripped serum has been used for preparing the standards. In addition, use of estradiol in conjugate buffer for displacing testosterone from SHBG from serum samples has been carried out. The use of this combination may possibly eliminate the matrix interference in other steroid assays.

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DOES BETTER REPRODUCTIVE HEALTH STATUS MEAN LOW FERTILITY LEVELS? EVIDENCE FROM ASIAN COUNTRIES

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ABSTRACT

In this paper, an attempt has been made to construct a composite index to describe reproductive health status of Asian countries and their fertility levels, based on eight indicators, mostly linked with maternity along with two other basic service indicators such as safe drinking water and sanitation. Two separate composite indices are also computed based on the two basic service indicators and the set of six maternity related indicators.

The analysis revealed that distribution of basic service index was more or less balanced which was not found to be true for the index of maternity. Also, there was a wide variation between ranks of index of maternity and basic service index. While considering the combined index of reproductive health based on indicators of maternal safety along with basic health indicators, it was found that better health index value does not necessarily go along with a lower level of fertility. Further, results of the analysis indicate that reproductive health status has least to do with the levels of fertility although an emphasis on reproductive health may result in fertility decline in due course.

Key-words: Fertility levels, Composite index, Reproductive health.

Fertility control being the central agenda in the anti-natalist population policies, the reproductive health orientation of such policies is hard to be realized without including fertility control. The recent emphasis on reproductive health as the prime key to population policies has been resulting in misleading connotations of reproductive health which is not distant from that of reproduction[1]. The evolution of changing thrust in the family welfare programme has been from mere imposition of fertility control to the provision of better child survival and safe motherhood along with better quality of services in the

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provision of health services delivery system. However, considering the concurrent changing thrust in the programme, reproductive health orientation seems holistic in the sense that it covers all aspects of female well being and especially the maternal well being. Comprehensive reproductive health services, according to Germain et.al.[2] should focus on sexual health beyond pregnancy, contraception and abortion, treatment of reproductive tract infections (RTIs), gynaecological services, and child health care, that is, they should encompass services for women of all ages, including adolescents and women beyond the reproductive ages. This may be reproductive health services in a wider perspective, but in any case if the family welfare programme has to have a reproductive health orientation then it has to emphasize on reproductive well being more than achieving fertility control alone.

Reproduction as it concerns women alone, reproductive health needs must take into consideration all roles and functions that relate to reproduction in general. However, at the same time, it is difficult to summarise the reproductive health status in terms of a given set of parameters since it is a complex construct that does not lend itself to an easy measurement. The basic elements of reproductive health are: responsible reproductive/sexual behaviour, widely available family planning services, effective maternal care and safe motherhood, effective control of reproductive tract infections (including sexually transmitted diseases), prevention and management of infertility, elimination of unsafe abortion and treatment of malignancies of reproductive organs[3]. In other words, reproductive health assessment includes the basic health needs in addition to the emphasis on needs related to the reproductive process in general.

Composite Indices to Measure Reproductive Health

There have been attempts to construct indices that measure the relative position of the States of India vis-a-vis reproductive health[4]. Such measures are not without their limitations[1]. It may be difficult to obtain a single index that would consider all aspects of reproductive health within its scope. On the other hand, it is possible to look at reproductive health in terms of its components and construct indices that measure its different components. Such indices may be describing reproductive well being at different stages of the female life cycle such as adolescence, reproductive years, pre-menopausal and menopausal, etc. As relevant information on reproductive health indicators at different stages of the life cycle are not available as yet at a macro level, there remains an obvious limitation in terms of constructing a reproductive health index in its holistic perspective and to describe certain components like maternity or contraceptive choice, etc.

Composite indices are useful at a policy level especially for evaluation purposes because they serve to simplify complex issues. However, such indices are limited in the sense that they tend to indicate the relative position of each country/community vis-à-vis each other, and their use for effective policy is also

very limited. Policy prescriptions of a very general kind is only possible using composite indices. This is because policy guidelines have to be based on specific factors that may be the component of the index. Therefore, such indices should be used with caution keeping in mind that they are only the means to an end.

In the absence of any single parameter to describe reproductive health status, it is often assumed that lower fertility levels ensure better reproductive health and vice versa. But, ensuring better reproductive health does not necessitate fertility control rather it ensures safe and effective reproductive life. It is in this context, that an effort is made here with regard to presenting an index of reproductive health based on indicators available and labelled as reproductive health indicators.

OBJECTIVES

In this paper, an attempt has been made to construct a composite index to describe the reproductive health status of Asian countries and their fertility levels, based on eight indicators, mostly linked with maternity along with two other basic health indicators, such as safe drinking water and sanitation. Two separate composite indices are also computed based on the two basic service indicators as well as the set of six maternity related indicators, named as basic service index and index of maternity.

MATERIALS AND METHOD

This is an effort to validate the myth of coincidence between low levels of fertility and better reproductive health status based on data from 27 selected countries in the Asian sub-continent. For this, first an index of reproductive health status has been constructed which was split into two components based on twin indicators related to safe drinking water and sanitation and another related to the six maternity indicators.

It is extremely difficult to find reliable data on reproductive health on a country-basis as only a few countries have published a broad range of such indicators and others have a very few of them. There are also gross inconsistencies between different data sources including UN organisations like UNICEF and WHO. Also, there is high variability between estimates from different years indicating the likelihood of validity problems. After checking various sources including the 1997 Human Development Report published by UNDP, the Social Indicators of Development, 1995, published by the World Bank and the 'The State of the World's Children 1997' (WHO, 1997); a reliable set of reproductive health indicators have been adjudged by UNDP based on the 'Global Health for all Indicators' prepared by WHO[6]. These data sets are found to be more consistent, reliable and up-to-date. They are in terms of safe water and sanitation, levels of fertility and contraceptive prevalence, levels of

female literacy together with maternity related characteristics like birth weight, pre-natal care, handling of deliveries, maternal mortality and anaemia during pregnancy. The time reference for all of these indicators across countries though not the same but can be considered to the nineties. Although, this set of indicators may be a partial list of all possible indicators describing reproductive health, it definitely describes the extent of safety with regard to maternity which is crucial for better reproductive health. These indicators exhibit a wide range of variation across nations irrespective of their levels of fertility and contraceptive prevalence. And for this reason, it is deemed desirable to exclude the fertility level as a parameter while assessing the relative position of a set of countries with regard to reproductive health status. Again, while deciding on the set of indicators for computing a summary index, all of them are made to follow the linearity assumption in a strict sense. It means that the two extremes for each indicator should conform to a best and worst situation in terms of 0 and 100 if they are expressed in percentage terms.

There has been no control over selection of these indicators chosen for computing a composite index of reproductive health status for a simple reason that these data are labelled as reproductive health indicators. At the same time, the selected set of indicators need to be described in terms of their bearing on reproductive health. All available literature provides evidence with regard to the basic services like safe drinking water and sanitation influencing the health status of a population in general. Therefore, these two indicators are included here. Besides these two indicators, all the other six indicators are directly linked with the reproductive aspect of women. Use of contraception indicates the extent of fertility regulation in the population which can always help avoiding unwanted and untimely pregnancies. Pre-natal care and birth care are the indicators describing the extent to which maternal safety is guaranteed in terms of ensuring safe obstetric outcomes.

Anaemia is a frequent abnormality found in case of pregnant women which is indicative of lack of maternal preparedness as well as proper maternal care in terms of preventives as well as nutrition. Pre-natal care should also include measures to avoid anaemia during pregnancy. Anaemia during pregnancy may affect both mother and the child. On the whole, it has both health and obstetrics implications.

Pre-natal care and birth care are defined in terms of measures adopted during pregnancy and delivery being carried out by professionals like doctors or trained birth attendants. These two indicators ensure safe maternity and least complications in post-partum situations.

Another maternal outcome indicator like proportion of low birth weight babies provides a clue regarding how well the entire course of maternity period has been dealt with, including various safety measures adopted not only to avoid maternity risks but also to have healthy children. In addition, maternal

mortality rate provides a direct assessment of maternal risk which must be resulting due to lack of precaution during maternity or lack of proper health services.

METHODOLOGY

The objective of this index is to represent the selected Asian countries in relative terms of reproductive health status in a range of values between 0 and 1 with a lower value representing better status in relation to a higher value of the index. A simple computation of the index is made by transforming each of the indicator values as a ratio of the difference between each value and the available best value to the entire range of variation in each of these indicators. This provides the relative positioning of the countries with respect to each of the selected indicators in a range of values between 0 and 1. Following which, an weighted average is computed for eight of the selected indicators, where the weights are the reciprocal of the corresponding coefficient of variations in each of the indicators. Such a weighted average is very much needed to make a sound index as each of these indicators has different extent of variation across States.

If X_{ii} is the jth indicator for the ith country, the transformed variable D_{ij}= Modulus (best (X_{ij})-X_{ij}) for all j = 1….8, where the best X_{ij} is decided depending on the concerned indicator's lower or higher value corresponding to the best situation.

Finally, the index value for the ith country is given by

$$Index_I = (Sum(D_{ij} * W_j)/(Sum(W_j) \text{ for all } j = 1…..8.$$

Where W_j is the reciprocal of the coefficient of variation of the jth indicator.

DISCUSSION

Level of Index of reproductive health status presented in Table 1 shows that among the 27 Asian countries selected for computation, Singapore ranks first with the lowest index value of .038 and India ranks last with the index value of .813. However, there seems to be a greater variation in the index values with one third of countries having an index value of more than 0.5 and another nine countries having an index value between 0 and 0.2. This shows the disparity in reproductive health status between countries as countries with better index values have the least dispersion against those with poor levels of reproductive health.

TABLE 1

INDEX OF REPRODUCTIVE HEALTH, INDEX OF BASIC SERVICES AND INDEX OF MATERNITY AND TOTAL FERTILITY RATES FOR SELECTED ASIAN COUNTRIES

Countries	Index of Rep. Health (RHI)	Rank (RHI)	Index of Basic Services (BSI)	Rank (BSI)	Index of Maternity (MI)	Rank (MI)	Total Fertility Rate	Rank TFR
Afghanistan	0.764	26	1.000	27	0.926	27	6.9	19
Bangladesh	0.678	23	0.420	15	0.739	24	3.4	10
China	0.179	8	0.705	25	0.146	6	1.9	25
India	0.813	27	0.557	19	0.767	25	3.4	23
Indonesia	0.425	17	0.460	17	0.601	20	2.9	3
Iran	0.225	11	0.258	10	0.419	16	5.3	17
Iraq	0.509	19	0.645	21	0.447	18	5.7	9
Israel	0.095	3	0.138	6	0.066	3	2.9	2
Japan	0.048	2	0.099	5	0.012	2	1.5	27
Jordan	0.212	10	0.380	13	0.263	10	5.6	1
Laos	0.601	22	0.698	24	0.709	23	6.7	13
Malaysia	0.136	6	0.094	4	0.163	8	3.6	4
Mongolia	0.128	5	0.307	12	0.071	4	3.6	14
Myanmar	0.576	20	0.659	22	0.355	14	3.6	26
Nepal	0.732	25	0.687	23	0.883	26	5.4	7
Oman	0.275	13	0.414	14	0.264	11	7.2	12
Pakistan	0.679	24	0.572	20	0.652	22	5.5	5
Philippines	0.291	14	0.216	8	0.429	17	4.0	11
Rep. of Korea	0.114	4	0.073	2	0.157	7	1.6	24
Saudi Arabia	0.154	7	0.279	11	0.139	5	6.4	15
Singapore	0.038	1	0.000	1	0.003	1	1.8	6
Sri Lanka	0.429	18	0.434	16	0.248	9	2.2	16
Syrian Rep	0.300	15	0.279	11	0.405	15	4.7	22
Thailand	0.187	19	0.186	7	0.343	13	1.9	20
Turkey	0.229	12	0.080	3	0.536	19	2.7	18
Vietnam	0.342	16	0.758	26	0.287	12	3.4	8
Yemen	0.583	21	0.533	18	0.643	21	7.6	21

Note: Data used for computing indices are from WHO (1997)[6].

APPENDIX TABLE

VARIABLES USED FOR COMPUTATION OF INDICES

Countries	Safe Water (1)	Anaemia (2)	Sanitation (3)	CCU (4)	B C (5)	PNC (6)	Birth Weight (7)	MMR (8)
Afghanistan	10.0	25	8	11.7	9.0	10.0	80	1000
Bangladesh	83	49	30	46	7	15	73..3	468
China	46	63	21	83	95	90	94	80
India	63	12	29	41	32	40	67	376
Indonesia	63	26	51	55	40	65	86	425
Iran	83	50	67	65	70	45	92	50
Iraq	45	50	36	14	60	45	82.4	50
Israel	99	75	70	65	99	90	92	5
Japan	95	80	85	59	100	99	93.8	18
Jordan	89	50	30	35	87	80	93	60
Laos	41	38	30	19	20	25	82	300
Malaysia	90	66	93.7	48	98	75	91.7	20
Mongolia	53.7	74	100	38	99	98	93.6	238
Myanmar	39	42	42	17	70	80	76.5	100
Nepal	48	25	22	23	6	10	76.8	515
Oman	56	50	72	9	75	89	91	20
Pakistan	60	43	30	12	35	25	75	400
Philippines	84	52	75	40	55	60	89	280
Rep. of Korea	89	63	100	79	89	90	91	30
Saudi Arabia	76	69	78	13.6	90	85	94	18
Singapore	100	82	100	67	99	99	92.6	4
Sri Lanka	57	38	66	66	94	97	78.2	40
Syrian Rep	87	48	56	36	61	65	89	180
Thailand	80.5	48	87	74	66	75	91.6	16
Turkey	91.7	26	94.4	63	76	45	92	180
Vietnam	38	37	21	65	90	95	88.2	200
Yemen	52	50	51	7	31.6	40	80.6	1000

Source: WHO (1997)[6]

Notes for the Appendix Table 1:

Col. 1: The information titled Safe Water refers to the percentage of population with safe drinking water available inside home or with reasonable access.

Having this observation in mind, the index was made into two components, one based on the twin indicators related to safe water and sanitation and another based on the six maternity related indicators. These two indices are named as basic service index and index of maternity. In relation to the basic service index, Singapore ranks first in order with the lowest index value of 0 and Afghanistan ranks at the bottom with an index value of 1.00. However, in this case the distribution of index values are more or less balanced with the first nine countries having index values ranging between 0 and .256 followed by the index value of next nine countries between .258 and .533.

In terms of the index of maternity, the two extremes are represented by Singapore and Afghanistan. But in this case, two third of the countries are within an index value ranging between 0 and 0.447 with the other nine countries dispersed within a range of 0.448 and 0.926. But there is a wide variation

between ranks of the basic service index and the index of maternity even though the extremes match each other.

While considering the combined index of reproductive health based on the indicators of maternal safety along with the basic health indicators, it is found that better index value does not necessarily go along with a lower level of fertility. It can be seen from Table 1 that better ranking countries like Singapore, Japan and Korea do have a below replacement level of fertility along with countries like Israel, Mongolia and Malaysia showing a better RHI value with a reasonable higher level of fertility. Again on the other extreme, countries like Saudi Arabia, Jordan and Oman exhibit a better reproductive health status despite having levels of TFR above 5.5.

In Table 1, rank correlation test is also applied to see the relationship between ranks given to reproductive health and fertility in respect of different countries. The rank correlation co-efficient value (.053) is insignificant at 5 per cent level of significance and 25 d.f.

Such a comparison ensures that reproductive health status has least to do with the levels of fertility, although an emphasis on reproductive health may result in fertility decline in due course. Such a comparison is made to eliminate the myth of the coincidence between lower fertility levels and better reproductive health.

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DOES CYCLIC ADENOSINE 3'-5' MONOPHOSPHATE ACT AS A REGULATOR FOR OOCYTE MEIOTIC RESUMPTION IN MAMMALS?

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ABSTRACT

Cyclic adenosine 3'-5' monophosphate (cAMP) acts as a second messenger for various pituitary hormones. The mode of involvement of cAMP in the regulation of meiotic maturation is not yet clear, because, different authors give evidences for both stimulatory and inhibitory actions of this nucleotide. Gonadotropic hormone induces meiotic resumption through signal transduction pathway involving cAMP. A growing body of evidence has generated several discrepancies for the actions of cAMP in both somatic cells and associated oocyte during induction of meiotic resumption. In the present article, the author has made an attempt to resolve the various contradictions by providing possible molecular explanations for the actions of cAMP during meiotic resumption in mammalian oocytes.

Key-words: Oocyte, Resumption of meiosis, Gonadotropin, cAMP.

Mammalian oocytes rest in the first meiotic prophase for a long time, i. e., from birth to puberty and are called dyctiate stage or germinal vesicle (GV) stage oocytes[1,2]. These fully-grown but immature oocytes are maintained at GV stage in follicular environment due to flow of an inhibitory level of cAMP from follicular cells to oocytes[3]. Resumption of meiosis is induced if the oocytes are removed from follicular environment and cultured in vitro or exposed to pituitary gonadotropin in species such as rat, mouse, rabbit, goat, bovine, human, etc[1,4]. The exact molecular mechanism(s) by which gonadotropin induces meiotic resumption is poorly understood. Studies using in vitro culture techniques have demonstrated that gonadotropins induce meiotic resumption through adenylate cyclase-cAMP pathway[1]. Short exposure of derivatives of cAMP or drugs, which are known to elevate intracellular cAMP levels, mimic the action of gonadotropin and induce maturation of rat cumulus-enclosed oocytes in vitro[1,3,6]. In contrast, the continuous exposure of these cAMP-elevating drugs inhibits meiotic resumption in vitro[1,3,6,7]. Further, the contradictory proposals for the action of cAMP during resumption of meiosis have been provided. In lower mammals, such as rodents, a transient decrease of cAMP level in the oocytes induces resumption of meiosis[9,10]. In addition, rodent oocytes do not possess adenylate cyclase system, therefore, oocytes are unaffected by the exposure of adenylate

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cyclase activator (cholera toxin) or gonadotropin[3]. In contrast, oocytes of higher mammals possess adenylate cyclase system and are capable to generate inhibitory levels of cAMP needed for the maintenance of meiotic arrest[9]. Further, an increase of cAMP in response to cAMP elevating drugs or gonadotropin disrupts gap junctions thereby reducing the flow of cAMP from somatic cells to oocytes. This leads to a transient decrease of cAMP during resumption of meiosis in rat oocytes[3]. In higher mammals, a continuous higher than the basal level of ooplasmic cAMP is responsible for the maintenance of meiotic arrest whereas a transient increase in its level facilitates resumption of meiosis in pig oocytes[9]. Hence, to resolve these contradictions, a generalized satisfactorily molecular explanation for the involvement of cAMP in regulation of meiotic resumption that suits to all mammalian species is required to be explored. For this purpose, simple hypothetical models could help to explain the maintenance of meiotic arrest or its resumption in preovulatory oocytes. In this review, an attempt is made to summarize, update and develop a better understanding of the role of cAMP during meiotic resumption in mammals.

Dual Role of cAMP for Resumption of Meiosis

Several in vivo and in vitro studies have demonstrated that cAMP play a dual role, i.e., inhibition and induction of meiotic resumption by modulation in its level in both cumulus cells and associated oocyte.

(I) ***cAMP as an Inhibitor of Resumption of Meiosis:*** cAMP plays a negative role during meiotic resumption[1]. This is supported by the fact that the continuous exposure of membrane permeable analogs of cAMP such as db-cAMP or 8-bromo-cAMP and other chemical compounds, which are known to elevate intracellular cAMP, also inhibit resumption of meiosis in rat, mouse, rabbit, goat and bovine oocytes in vitro [3,6,11,12]. Nevertheless, the continuous transfer of an inhibitory level of cAMP from cumulus cells to oocytes is responsible for the maintenance of meiotic arrest in rodents[3]. There are two possibilities for the generation of an inhibitory level of cAMP in the ooplasm. The first possibility is an inhibition of type-3 phosphodiesterase (type 3 PDE) that hydrolyzes ooplasmic cAMP spontaneously. Second possibility is that the oocyte receives a tonic level of cAMP from associated cumulus cells through gap junctions. In higher mammals, oocyte possesses an adenylate cyclase system that generates cAMP sufficient to inhibit meiotic resumption. Taken together, these studies confirm a negative role of cAMP during regulation of meiotic resumption in mammals.

(II) cAMP as an Inducer of Resumption of Meiosis**:** In rodents, an initial decrease in the level of oocyte cAMP is prerequisite for resumption of meiosis [6,8,11,13]. This initial decrease in the level of oocyte cAMP may be due to activation of cAMP-PDE[8] or disruption in the communication between cumulus cells and associated oocytes[3] in case of spontaneous maturation. The short exposure of membrane permeable analogs of cAMP such as db-cAMP or 8-bromo-cAMP induces meiotic resumption of cumulus-enclosed oocytes in vitro[3]. Similarly, other drugs which are known to elevate intracellular cAMP, also mimic the action of gonadotropin and induce resumption of meiosis in cumulus-enclosed oocytes in rodents[3,6].

The possibility for the induction of meiotic maturation by these drugs may be due to a transient increase in the level of cAMP in associated cumulus cells. This increased cAMP level then might have interrupted the flow of an inhibitory level of cAMP from follicular cells to oocyte resulting in the net decrease in ooplasmic cAMP level during meiotic resumption. On the other hand, studies in higher mammals show that, unlike rodents, an increase in the oocyte cAMP compared to its basal level is associated with resumption of meiosis in rabbit[14]. Later, Mattioli et al[9] documented, in an elaborated study, that the continuous higher level of ooplasmic cAMP is responsible for maintenance of meiotic arrest and a transient increase in its level may facilitate meiotic resumption in pig cumulus-enclosed oocyte. In porcine, exposure of cAMP derivative (db-cAMP) increases both nuclear and cytoplasmic maturation of oocytes[15]. In bovine, the maintenance of higher level of cAMP during in vitro maturation promotes developmental competency of oocyte[10]. This initial increase in the level of cAMP is probably due to internalization of gonadotropin receptors and thereby desensitization of follicular cells to gonadotropin. Another possibility is that a soluble factor, which originates from associated follicular cells, directly induces ooplasmic cAMP levels[9]. Recently, Modina et al[10] have observed that the cAMP induces cumulus cells expansion and cumulus cells-oocyte gap junctions during meiotic maturation in bovine. These observations clearly suggest that, unlike rodents, in higher mammals, a transient increase in the level of ooplasmic cAMP is associated with resumption of meiosis and a continuous higher level of cAMP maintain oocyte meiotic arrest.

Possible Mechanism of Regulation of Oocyte cAMP Levels

The action of gonadotropin on preovulatory oocytes to stimulate meiotic resumption may be due to increase of follicular cAMP level through adenylate-cyclase-cAMP mediated pathway in most of the mammalian species[1]. This conclusion seems to present an apparent paradox. If the treatment of exogenous cAMP inhibits resumption of meiosis in cultured rat oocytes[3,4], what mechanism allows oocytes to resume meiosis in vitro in response to gonadotropic hormones-elevated cAMP? Since both inhibition and induction of meiotic resumption could be enhanced in rodents by various cAMP levels. It may be hypothesized that the activation/inactivation of adenylate cyclase and cAMP-PDEs alone or their cumulative effects might be involved in the modulation of cAMP levels in cumulus cells and associated oocyte. The fluctuations in ooplasmic cAMP levels then regulate phosphorylation/dephosphorylation of cAMP dependent PKs. This phosphorylation/dephosphorylation of cAMP dependent PKs regulate MPF activity directly and ultimately regulate maintenance of meiotic arrest in mammals.

Upstream Regulation of cAMP Levels in Somatic Cells and Oocyte

(I) ***Modulation of cAMP Levels through Adenylate Cyclase Pathway:*** The adenylate cyclase is a membrane bound enzyme that catalyses the conversion of cytosolic adenosine triphosphate (ATP) to cAMP for most of the physiological activities in eukaryotic cells. Pituitary gonadotropin induce meiotic resumption in most of the mammalian oocytes through adenylate cyclase-cAMP- pathway[1,6]. Activators of adenylate cyclase induce meiotic

maturation in cumulus-enclosed but not denuded oocyte in rodents[1]. These findings indicate the availability of adenylate cyclase system at the level of cumulus cells and its activators mimic the action of gonadotropin to induce meiotic resumption in rodents. In contrast, denuded oocytes do not possess the adenylate cyclase system; therefore, resumption of meiosis is not induced after the exposure of gonadotropin or adenylate cyclase activators in rodents in vitro[3]. It is possible that maturing oocytes posses atypical adenylate cyclase at the level of oocyte consisting of only a single catalytic subunit and not coupled to regulatory G proteins sensitive to cholera toxin or pertussis toxin[13]. This may be a reason that rat and mouse oocytes do not elevate cAMP levels in response to gonadotropin, whereas, forskolin does increase cyclic nucleotide level and inhibit resumption of meiosis in rodents. It is possible that the inhibitory level of cAMP is synthesized by cumulus cells and transferred to oocytes via gap junctions[1,3]. Support for this possibility came from observations that when cAMP levels were high in cumulus-oocyte complex, cAMP content was also high in their associated oocyte[8]. Unlike rodents, an increased level of cAMP within the oocyte is documented at the time of resumption of meiosis in sheep[16], rabbits[14] and pig[9] oocytes, suggesting the existence of adenylate cyclase-cAMP system at the level of oocytes in higher mammalian species. This possibility was further strengthened by the observations that bovine oocytes posses adenylate cyclase system and generates sufficient amount of cAMP to maintain resumption of meiosis[17]. Later, using cytochemical technique, an adenylate cyclase system was localized at the level of oocytes of higher mammals[18] (fig.1). Taken together, these findings support the hypothesis that both compartments (i.e. cumulus cells or oocytes) possess adenylate cyclase-system to regulate cAMP and thereby resumption of meiosis in mammalian oocytes.

(II) ***Modulation of cAMP levels through cAMP-Phosphodiesterase (cAMP-PDE) pathway*****:** Gonadotropin-induced meiotic maturation or spontaneous maturation in cumulus- enclosed or denuded oocytes does not occur in the presence of inhibitors of cAMP-PDE in vitro[6]. These results suggest that the possible site for action for these inhibitors may be oocyte cAMP-PDE. An increase of PDE activity could hydrolyse cAMP and stimulate a transient decrease of oocyte cAMP level associated with resumption of meiosis[8]. Although cAMP-PDE is not altered during maturation of mouse oocyte in vitro, the observations that IBMX and theophylline inhibit both GVBD and associated decrease in the level of cAMP strengthen the conclusion that PDE inhibitors reversibly block GVBD by their action on oocyte-PDE[8]. These results indicate the involvement of cAMP-PDE in the regulation of oocyte maturation. In addition, evidences from non-mammalian species have also indicated that the decrease in th level of cAMP is associated with increased activity of oocyte of cAMP-PDE during meiotic resumption[19,20] . Both theophylline and IBMX reversibly block oocyte maturation by inhibiting PDE activity leading an accumulation of cAMP levels in fish oocyte[21]. Later, we have also demonstrated that the treatment of caffeine and pentoxifylline (other inhibitors of PDE) prevent resumption of meiosis both in cumulus-enclosed as well as denuded rat oocytes in vitro[7]. The existence of various forms of cAMP-PDEs has been documented during resumption of meiosis. These different forms of cAMP-PDEs are highly compartmentalized. A study using

selective inhibitors has shown that type 3 PDE, which is cAMP specific, is located in the cytoplasm of oocytes. The activation of type 3 PDE inhibit both gonadotropin-induced and spontaneous maturation of rat oocytes in vitro[6,7,22,23]. On the other hand, type4 PDE is specifically located in the somatic compartment associated to oocytes. The inhibitors of type 4 PDE mimic the action of gonadotropin and induce meiotic resumption in cumulus-enclosed oocytes (fig.2). However, these inhibitors do not affect the maturational status of denuded oocytes in vitro[23]. The paradoxical actions of PDEs may regulate the meiotic maturation by generating preferential fluctuations of cAMP in both cumulus cells and corresponding oocyte. Further, to strengthen this hypothesis, using in-situ hybridization studies, it has been demonstrated that the PDE3B mRNA is concentrated in the oocyte cytoplasm, whereas PDE4D is located in the somatic cells associated to oocyte[23]. These observations support the hypothesis that selective expression of both PDEs in various compartments (somatic and germ cell) modulate cAMP levels and thereby regulate meiotic resumption in mammalian oocytes.

Downstream Regulation of cAMP Levels in Somatic Cells and Oocyte

Role of cAMP-dependent Protein Kinases (cAMP -PKs): Cyclic AMP- dependent protein kinases consist of catalytic © and regulatory ® subunits. The complex form of cAMP-PKs are inactive and the association of cAMP with its ® subunit results in the dissociation and activation of © subunit. It is now accepted that cAMP-PKs could regulate phosphorylation / dephosphorylation of protein (s) that are associated with meiotic resumption of oocytes[1,24]. Two cAMP-PKs (PKA and PKC) are probably involved in the regulation of meiotic resumption. PKA is associated with the phosphorylation of protein(s) necessary for the maintenance of meiotic arrest in hamster oocytes[25,26]. A high-sustained level of PKA activity in the oocyte inhibits meiotic resumption and a transient increase in its level induces resumption of meiosis in mouse oocytes[27]. Further, it has been proposed that the PKA activity is highly compartmentalized. This is supported by the fact that the activation of intraoocyte PKA type-I inhibits spontaneous maturation, whereas the activation of type-II PKA in follicular cells induces meiotic resumption[28] (fig.3a). Another kinase that may play important role in the regulation of meiotic resumption is PKC[29]. Elevation of PKC activity has also been observed during gonadotropin-induced resumption of meiosis in preovulatory follicles[29]. Whether this increased PKC activity is involved in gonadotropin-induced meiotic resumption of meiosis is questionable. PKC activators have been shown to prevent meiotic resumption in mouse[30], bovine[26] and porcine oocytes[31]. In contrast, PKC can stimulate meiotic resumption in mammalian oocytes[14,29,32]. Further, evidences are provided for role of PKC specifically during G_2/ M border of the meiotic cell cycle. PKC activity appears at prophase-I and increases with respect to progress of meiotic maturation. The enzyme activity is the highest at M-I arrested oocytes. The involvement of PKC during metaphase-I/metaphase-II (M-I/M-II) transition has also been documented in mouse oocytes[32]. Loss of PKC activity disrupts the critical M-I to M-II transition, leading to a precautious exit from meiosis. Further, using an in vitro culture system, Lu, et al.,[27] demonstrated that inhibition of meiotic maturation is mediated through PKC-dependent pathway. Similar to PKA, PKC is also compartmentalized during meiotic maturation. Activation of PKC in the cumulus cells

generate stimulatory factor that induces meiotic resumption. However, PKC activation in the cytoplasm of oocytes inhibits spontaneous maturation (fig.3b). Taken together, these evidences support the hypothesis that both PKA and PKC regulate meiotic resumption either alone or by their cumulative effect through cAMP-dependent pathway.

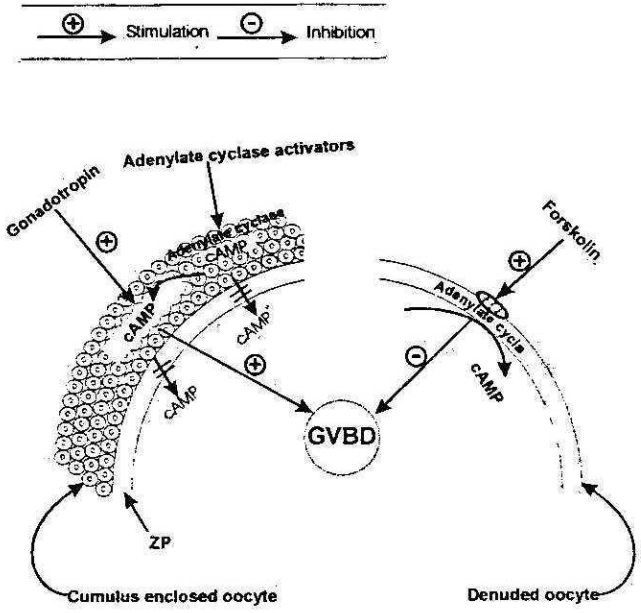

Fig.1: Proposed model for the regulation of meiotic resumption by adenylate cyclase in cumulus enclosed and denuded oocytes.

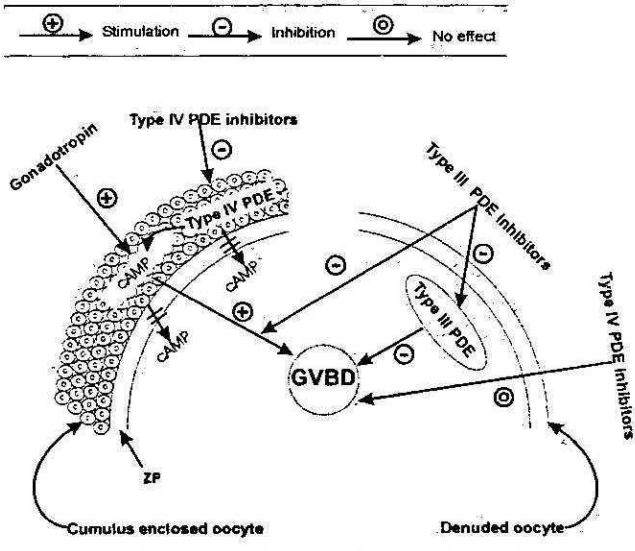

Fig.2: Proposed model for the regulation of meiotic resumption by cAMP-PDEs. Selective localization of both type-III PDE and type-IV PDE in oocyte and associated cumulus cells,respectively

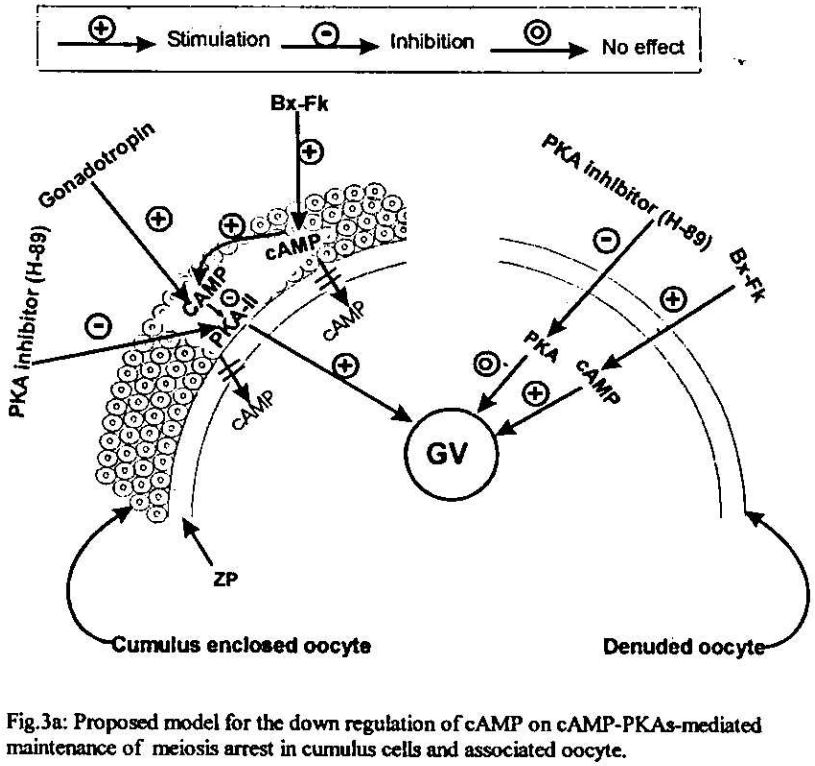

Fig.3a: Proposed model for the down regulation of cAMP on cAMP-PKAs-mediated maintenance of meiosis arrest in cumulus cells and associated oocyte.

REGULATION OF MOUSE OOCYTE MATURATION BY PROTEIN KINASE C

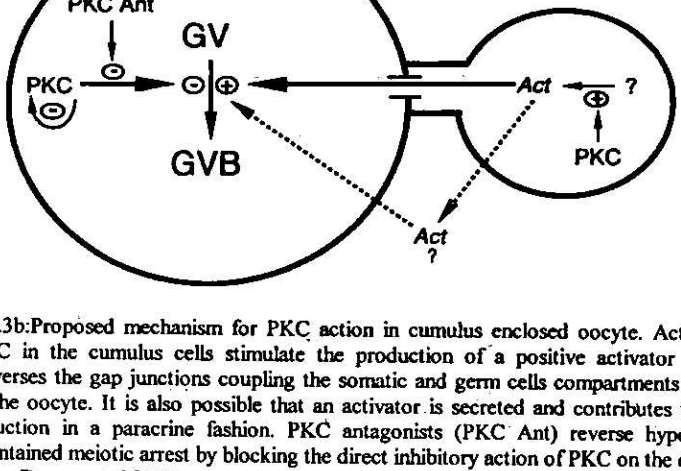

Fig.3b:Proposed mechanism for PKC action in cumulus enclosed oocyte. Activation of PKC in the cumulus cells stimulate the production of a positive activator (act) that traverses the gap junctions coupling the somatic and germ cells compartments to trigger in the oocyte. It is also possible that an activator is secreted and contributes to meiotic induction in a paracrine fashion. PKC antagonists (PKC Ant) reverse hypoxanthine-maintained meiotic arrest by blocking the direct inhibitory action of PKC on the oocyte. From Downs et al,2001.

CONCLUSION

The contradictory action of cAMP in the regulation of meiotic maturation is due to its cellular compartmentalization in cumulus oocyte complexes. An increase of cAMP level in the follicular cells induces meiotic resumption, while, in oocyte inhibits resumption of meiosis. Gonadotropic hormone elevates cAMP level in the follicular cells associated to oocyte. This elevated cAMP level disrupts the gap junctions within the cumulus cells or between cumulus cells and oocyte. The disruption in the gap junctions then reduces flow of cAMP from follicular cells to oocyte leading to a transient decrease of ooplasmic cAMP level. This initial decrease of ooplasmic cAMP level regulates the phosphorylation /dephosphorylation of cAMP–PKs responsible for MPF activation. Once the MPF is activated in the ooplasm, inhibition is relieved and meiosis is resumed. On the other hand, a high-sustained level of cAMP within oocyte prevents meiotic resumption. Thus, the fluctuations of cAMP levels in various compartments (follicular cell and oocyte) play critical role in the regulation of meiotic maturation. This fluctuation of intracellular cAMP levels is regulated by activation/inactivation of adenylate cyclase, cAMP-PDF either alone or in combination. Taken together, these findings support the hypothesis that cAMP is a key regulator for resumption of meiosis in mammalian oocytes.

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KNOWLEDGE AND ATTITUDE OF SCHOOL STUDENTS ABOUT POPULATION RELATED ISSUES

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ABSTRACT

The findings of the study revealed that (i) the students had low level of knowledge and less favourable attitude towards population related issues; (ii) the Self Instructional Guidelines (SIG) was found to be effective in enhancing the knowledge and developing more favourable attitude towards population related issues; (iii) the mean-knowledge score of rural boys about population related issues increased from 30.7 in the pre-test to 39.5 in the post-test, and their mean attitude score increased from 104.8 in the pre-test to 118.1 in the post-test; (iv) the mean difference between pre-test and post-test attitude scores for urban boys was 8.5, and for rural boys it was 13.3; similarly, the mean attitude score of urban girls raised from 105.6 in the pre-test to 113.5 in the post-test; and for the rural girls it increased from 97.9 in the pre-test to 115.2 in the post-test; and (v) before the administration of SIG, the rural boys got the highest mean score in knowledge followed by the urban girls, urban boys and rural girls. The author suggests that efforts in the area of population education may be intensified by including it in the school curriculum.

Key-words: Self Instructional Guidelines (SIG), Population related issues.

Population explosion has become one of the most fundamental human problems in the recent years in the world and it has become one of the major challenges for India. A look at the census data of the country shows that the population of India, which at the turn of the twentieth century was around 238.4 million, increased by more than four times in a period of hundred years, to reach 1,027 million at the dawn of the twenty first century. With only 2.4 per cent of the land area and 16 per cent of the world's population, India has the dubious distinction of being the second most populous country in the world. And if the current trend continues, India may overtake China to become the most populous country in the World by 2045[1,2]. The adolescents of today will be the adults and parents of tomorrow and their reproductive behaviour will play an important role in controlling the rate of population growth. So, they need to be educated at an

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early age about the importance of reproductive health and the size of the family. They are also expected to develop a favourable attitude towards population related issues. However, available data indicate that their knowledge about population related issues is not up to the mark[3,4,5,6,7,8,9,10,11]. The National Population Policy adopted by the Government of India in February 2000 lays a considerable emphasis on adolescents' health while discussing about the strategic themes and operational strategies for stabilising the population growth[2]. Therefore, the present study has been designed to understand the knowledge of the senior secondary school students (both boys and girls of urban and rural areas) about population related issues. Also, the study attempts to find out whether the students are having favourable attitude towards population related issues or not.

SPECIFIC OBJECTIVES

(i) To develop self instructional guidelines (SIG) on population education for senior secondary school students,

(ii) To assess the knowledge of senior secondary school students, both boys and girls of urban and rural areas before and after the administration of Self Instructional Guidelines (SIG) on population education,

(iii) To assess the attitude of senior secondary school students, both boys and girls of urban and rural areas before and after the administration of SIG on population education,

(iv) To compare the knowledge and attitude of senior secondary school students, both boys and girls of urban and rural areas, before and after the administration of SIG on population education,

(v) To establish relationship between knowledge and attitude of senior secondary school students, both boys and girls of urban and rural areas, before and after the administration of SIG, and

(vi) To determine the acceptability and utility of SIG by the senior secondary school students and teachers.

METHODOLOGY

The study was conducted in the month of January 2002. The sample consisted of 123 senior secondary school students, both boys and girls of XIth standard, who were drawn from four government schools of Delhi, representing two from rural and two from urban areas. The students were selected by using a multi-stage cluster sampling technique, and zone and school were selected by following the convenient sampling method. The urban area selected for the study was Sarojini Nagar and the rural area was Ujwa-Najafgarh.

A knowledge questionnaire having 46 items, a five-point likert type of attitude scale with 26 statements, and an opinionniare having ten items were developed and utilized for data collection.

Chronbach alpha and KR-20 formulae were used to establish the reliability of the tools and they were found highly reliable, having the reliability coefficient of .80, .82 and .91 respectively. The independent variable in the study was Self Instructional Guidelines (SIG) and the dependent variables were the scores on knowledge test, attitude test and opinionniare for acceptability and utility of SIG.

Content validity of the tools and SIG were worked out by administering them to nine experts, including five from the field of nursing, two from community medicine and two from education. Their suggestions were incorporated in the tools and guidelines were modified accordingly.

The pre-test and SIG were given on day one. Post-test and opinionniare were given on 8th day of the study.

Self Instructional Guidelines (SIG)

Self instructional guidelines in the present study refers to the self sufficient unit of written instructions on population education used for teaching senior secondary school students.

For developing self-instructional guidelines, the following subjects were considered:

(i) demographic aspects,
(ii) causes and consequences of population explosion
(iii) possible ways of controlling population growth
  (a) empowerment of women and gender equality,
  (b) reproductive health issues,
  (c) family life education and
  (d) family planning.

FINDINGS

Knowledge Level of Senior Secondary School Students

The students were having low level of awareness of population related issues. The rural girls got the lowest mean score (26.8) followed by the urban boys (29) and urban girls (29.6). Interestingly, the rural boys got the maximum mean pre-test score (30.7).

The findings of the study also revealed that the mean post-test knowledge score of students after the administration of SIG was significantly higher than their mean pre-test score. This is evident from the t-test, that SIG was effective in increasing the awareness of population related issues among students. With regard to population issues, the study observed an increase in

the knowledge level of both the boys and girls of urban and rural areas. While the pre-test and post-test mean knowledge difference was 7.2 for urban boys and 7.7 for urban girls. On the same line, knowledge level of rural boys increased from 30.7 in the pre-test score to 39.5 in the post-test score, and for rural girls it increased from 26.8 to 35.2 (Table 1).

TABLE 1

MEAN, MEAN DIFFERENCE, T-VALUE OF PRE-TEST AND POST-TEST KNOWLEDGE SCORES OF URBAN AND RURAL SENIOR SECONDARY SCHOOL BOYS AND GIRLS

N=123

Group	***Mean knowledge scores***		***$Mean_D$***	***t-value***
	Pre-test	*Post-test*		
Urban Boys (n=30)	***29.0***	***36.2***	***7.2***	*13.58* **(df-*29*)**
Urban Girls (n=32)	***29.6***	***37.3***	***7.7***	*10.3* **(df-*31*)**
Rural Boys (n=31)	***30.7***	***39.5***	***8.8***	*19.13* **(df-*30*)**
Rural Girls (n=30)	***26.8***	***35.2***	***8.4***	*21.53* **(df-*29*)**

t (30) = 1.70, p <.05 ***t(31) = 1.70, p<.05*** ***t(29) =1.70,p <.05***

Attitude of Urban and Rural Senior Secondary School Students

The students were having less favourable attitude towards the population related issues. After undergoing the SIG, a significant change was observed in their attitude.

The mean post-test attitude scores of the students were found significantly higher than their mean pre-test attitude scores at .05 level of significance. It means the SIG was effective in developing more favourable attitude towards population related issues. The mean difference between pre-test and post-test attitude scores for urban boys was 8.5; and for rural boys it was 13.3. Similarly, the mean attitude score of urban girls raised from 105.6 in the pre-test to 113.5 in the post-test; and for the rural girls it increased from 97.9 in the pre-test to 115.2 in the post-test (Table 2).

TABLE 2

MEAN, MEAN DIFFERENCE, AND T-VALUE OF PRE-TEST AND POST-TEST ATTITUDE SCORES OF URBAN AND RURAL SENIOR SECONDARY SCHOOL BOYS AND GIRLS

N=123

Group	*Mean Attitude Scores*		*Mean$_D$*	*t-value*
	Pre-test	***Post-test***		
Urban Boys (n=30)	***100.5***	***109***	***8.5***	*8.20* **(df-*29*)**
Urban Girls (n=32)	***105.6***	***113.5***	***7.9***	*5.2* **(df-*31*)**
Rural Boys (n=31)	***104.8***	***118.1***	***13.3***	*13.74* **(df-*30*)**
Rural Girls (n=30)	***97.9***	***115.2***	***17.3***	*13.62* **(df-*29*)**

t (30) = 1.70 p <.05 ***t(31) = 1.70 p<.05*** ***t(29) =1.70 p <.05***

Knowledge and Attitude Test Scores of Senior Secondary School Students

Before the administration of the SIG, the rural boys got the highest mean score in knowledge followed by the urban girls, urban boys and rural girls. However, after applying statistical test, the results revealed that the mean pre-test knowledge and attitude score of rural boys and urban girls were found to be significantly higher than the rural girls at 0.05 level of significance. After the administration of SIG, the rural boys and girls demonstrated a higher gain of knowledge than the urban boys and girls. The pre-test and post-test mean attitude scores of urban boys were 100.5 and 109 respectively. In case of urban girls, the scores were 105.6 and 113.5 respectively. For rural boys and girls, the scores were 104.8 and 118.1, and 97.9 and 115.2 respectively. The mean gain in knowledge score of rural boys was found significantly higher than the urban boys at .05 level of significance. Further, mean gain in attitude score of the rural girls was found to be significantly higher than the rural boys and urban boys. Mean gain in attitude score of the rural boys was found significantly higher than the urban boys at .05 level of significance.

Correlation between Knowledge and Attitude

After the administration of SIG, a significant positive correlation was observed between the knowledge and attitude scores of senior secondary school students (Table 3). The r value between the post-test knowledge and attitude scores of urban boys is .35 which is close to the table value but found to be statistically not significant. The r values in Table 3 further show that SIG was effective in enhancing the knowledge as well as developing more favourable attitude towards population related issues by the senior secondary school students.

TABLE 3
CO-EFFICIENT OF CORRELATION BETWEEN POST-TEST KNOWLEDGE AND POST-TEST ATTITUDE SCORES

Group	***R***
Urban Boys (n-30)	**0.35 (*df-*28)*
Urban Girls (n-32)	*0.38 (*df-*30)*
Rural Boys (n-31)	*0.76 (*df-*29)*
Rural Girls (n-30)	*0.38 (*df-*28)*

r(28)= 0.361, p<.05 r(29)= 0.355, p<.05 r(30)=0.349, p<.05

Acceptability of Self Instructional Guidelines (SIG)

The opinions about the acceptability and utility of SIG were collected from 123 students and teachers of senior secondary schools. The mean scores of senior secondary school students and teachers on acceptability and utility of SIG were 28.5, 28.4, 28.8, 28.7 and 28 for urban boys, urban girls, rural boys, rural girls, and teachers respectively. This suggests that both students and teachers accept SIG as one of the effective methods of teaching.

DISCUSSION

The urban and rural senior secondary school students, both boys and girls, had a low level of knowledge and less favourable attitude towards population related issues before the administration of self instructional guidelines (SIG), the study revealed. The findings of this study are to some extent in consistent with the findings of V.K. Srivastava[6]. In addition, the findings of the present study are also in agreement with the findings of K.M. Mariam who opined that majority of the students found to have inadequate knowledge about issues like status of women, marriage, family life and family planning[14]. Low level of awareness of population growth hazards among students has also been observed[4,9,15].

The use of self instructional guidelines (SIG) has been found to be effective in enhancing the knowledge of students about population related issues. In the present study also, the administration of SIG has helped the students to acquire knowledge about population related issues as well as to develop favourable attitude towards population related issues. This finding is in agreement with the findings of B.S. Mishra[13]. In his study, Mishra suggested that educational material was found to be effective in increasing the awareness of population problem among senior secondary school students. Some other studies have also supported the application of SIG[3,12].

In the present study, an attempt was made to compare the knowledge level as well as attitude between the boys and girls of urban and rural areas. A significant difference between urban and rural girls was observed with regard to

knowledge and attitude, but no significant difference was observed between urban and rural boys. The findings of the study are contradictory to the findings of Population Education Cell, Bihar[9].

With regard to attitude, it was found that rural senior secondary school students had better attitude score than the urban senior secondary school students. This is in agreement with the study findings of M. George[16]. This is probably due to the fact that rural senior secondary school students may be more influenced by their parents and they tend to listen to them.

IMPLICATIONS

The findings of the study imply that our educational system may have to be tuned to meet the issues of population problems in general. In this process, in addition to parents, the school teachers play an important role. It would be appropriate if the teachers at the school level are given an orientation training in health and population education. The teachers' attitude towards population related issues is also equally important.

LIMITATIONS

Due to the time factor, the study was limited to only one group and no control group was considered in the study.

CONCLUSION

Population problem is a matter of great concern and is closely related to development. The observations of the present study clearly indicate that the school going adolescents have a low level of knowledge and less favourable attitude towards population related issues. Adolescents of today are the prospective parents of tomorrow. They are expected to have adequate knowledge and desirable attitude towards population issues in general. This will result in stabilising the population growth in the country. Therefore, the author suggests that efforts in the area of population education may be intensified by including it in the school curruculum. This will facilitate the young adults to realise the importance of health education in their life situation.

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PREFERENCES FOR HOME DELIVERIES IN A SUB-URBAN COMMUNITY OF BANGALORE CITY

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ABSTRACT

The study attempts to find out the preferences of women belonging to a sub-urban community of Bangalore city either for home deliveries or institutional deliveries and tries to correlate the relationship between the factors such as the educational status and socio-economic status of women and their preferences. In addition, the influence of the place of delivery on the care of the newborn and infant is also investigated in this study.

The study revealed that (i) educated parents and families belonging to a higher socio-economic status preferred hospital deliveries; (ii) difference in the acceptance of ante-natal care between these two groups was not significant; and (iii) children born in hospitals found to have a better chance of being breast-fed within the first 30 minutes of delivery and were also more likely to be fully immunised in time as compared to those delivered at home.

Key-words: Home deliveries, Ante-natal care, Preferences.

Home deliveries are traditional, however, they are still in practice in some countries including India[1]. The National Health Policy of India (1982) suggested that "efforts should continue at providing refresher training and orientation to traditional birth attendants (TBAs); schemes and programmes should be launched to ensure that progressively all deliveries are conducted by competently trained persons so that complicated cases receive timely and expert attention, within a comprehensive programme providing ante-natal, intra-natal and post-natal care[2]." The policy set a goal to conduct 100 per cent deliveries by the trained attendants[2]. However, the situation in the country is different. Rajesh Kumar et al[4] observed in their study in Haryana that 76.1 per cent of the deliveries were conducted by the trained birth attendants (TBAs) in a sub-centre village followed by 75.6 per cent of the deliveries in villages without sub-centres

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and only 49.8 per cent of the deliveries in a Primary Health Centre village[3]. The findings suggest that availability of trained birth attendant in sub-centre villages helps the women to have her services for their deliveries. Similar observation was also made by Deivanayagam et al in their study in Tamil Nadu[4]. Their observation was that 73.7 per cent of the deliveries were domiciliary and conducted by dais[4]. In contrast, Doke and Sathe, in their study reported that 68 per cent of deliveries were conducted in institutions[5]. However, other studies have reported varying degree of preferences for home and institutional deliveries[6,7,8,9,10]. While reviewing the literature on the subject, it is clear that institutional deliveries are more common in developed world and home deliveries are more common in developing world[11].

Medical services are provided to the sub-urban community of Bangalore city by a private health centre (with mobile health facilities). Interactions with the women who attended the weekly 'mobile clinic' at Shikaripalya, located on the outskirts of Bangalore, led to the suspicion that the women in the area had a preference for home deliveries. Initial Key Informant Interviews with some of these women confirmed this view. Therefore, the investigators tried to find out the reasons for such a preference.

OBJECTIVES

(i) To identify the preferences of the married women, who had delivered children in the past five years, for either home or institutional delivery;
(ii) To understand the demographic factors, if any, which had a role to play in their choice of the place of delivery;
(iii) To assess the ante-natal services availed by the women who fell into either group of home delivery or institutional delivery; and
(iv) To find out the differences, if any, in the care of the new-born and infant between these two groups.

MATERIALS AND METHOD

A house-to-house survey of the community was conducted, which covered a total of 368 households. A pre-tested questionnaire was administered to all the mothers who had children of less than five years of age. In case of families where there were more than two children below the age of five years, the relevant parts of the questionnaire were repeated for each child. Data so collected were entered into the SPSS statistical package. The chi-square test was used to establish any significant association between the different variables and the outcome.

FINDINGS AND DISCUSSION

270 mothers had delivered 326 children in the past 5 years out of the 368 households, of which 132 (40.5%) deliveries took place in a hospital. Of the

remaining 194 (59.5%) deliveries, 119 (61.3%) were conducted by an untrained dai, 72 (37.1%) were conducted by the auxiliary nurse mid-wife and only 3 (1.5%) were conducted by a trained dai (Table 1).

TABLE 1
DETAILS OF DELIVERIES

No. of households	368
No. of households with children less than 5 years	270
No. of children less than 5 years	326
No. of children delivered at hospital	132
No. of children delivered at home	194
- Untrained traditional birth attendants	119
- Government ANMs	72
- Trained traditional birth attendant	3

The most common reason for preferring home deliveries by the women in the study area was the cost incurred in hospital deliveries, 47.9 per cent (Table 2). Obviously, in addition to direct expenditure on hospitalisation of a woman for delivery, the family has to spend some more money indirectly on transport, visits by the family members to the hospital, etc. While discussing with the women who had delivered at home, it was found that most of them preferred hospital as a safe place for delivery, but because of their economical conditions, they were forced to stay back at home for delivery. Further, they felt hospital delivery would be very expensive in terms of transport and their stay in the hospital. While having discussions with the TBAs, it was found that they did not demand any payment for their services. Further, it was also observed that home delivery was popular in the study area[13] and the women preferred the TBA to conduct the delivery irrespective of her skill. Sometimes, unexpected onset of labour in some women demanded home delivery[12]. The other reasons quoted by the respondents for home delivery were tradition 32.5 per cent, inconvenient time of delivery 4.6 per cent and un-availability of transport 4.6 per cent (Table 2).

TABLE 2
REASONS FOR HOME DELIVERY (n=194)

Reason for home delivery	**Number (Percentage)**
Cost of hospital delivery	93 (47.9%)
Tradition	63 (32.5%)
Unexpected onset of labour	20 (10.3%)
Inconvenient time of delivery	9 (4.6%)
Unavailability of transport	9 (4.6%)

Data presented in Table 3 reveal the reasons for preferring deliveries in the hospital by the respondents under the study. The major reasons opinioned by the women were problems during labour (33.3 %) followed by always utilised

hospital services (27.3%) and primi gravida (17.4%). The other reasons given by the women include ANC complications, sick during advanced pregnancy, etc.

Women about 27.3 per cent preferred hospital delivery who stated that they always utilised the hospital services for all of their problems and 17.4 per cent of them opinioned that they utilised the hospital services for the first delivery only. One customary practice in the study area is that if the delivery takes place in the pregnant women's mother's home means, it is likely to take place in hospital because they are prepared to pay any amount to ensure her safety, particularly for the first delivery.

TABLE 3
REASONS FOR HOSPITAL DELIVERIES (n=132)

Reasons for hospital delivery	**Number (Percentage)**
Problems during labour	44 (33.3%)
Always utilised hospital services	36 (27.3%)
Primi gravida	23 (17.4%)
Goes to mother's house	16 (12.1%)
Recognised ANC complications	7 (5.3%)
Fallen ill recently	6 (4.5%)

Table 4 shows that uneducated mother, 143 out of 223 and uneducated father 125 out of 191, preferred home deliveries in the study area. Mother's education had a little influence on hospital deliveries as compared to father's education. Similarly, families with a per capita income of less than Rs. 2,500/- preferred home deliveries. This reinforces the fact that poor families can not afford to have hospital deliveries. It is also clear from Table 4 that a larger proportion of those who had home deliveries availed of the antenatal services provided by the mobile team from the private health centre. However, it was seen that those who had not availed of the services, including tetanus toxoid immunisations and iron and folic acid tablets, were less likely to avail of hospital deliveries. In the case of post-natal services, it was observed that those who had delivered at the health centre, were initiated on breast-feeding after 30 minutes or were given pre-lacteal feeds. Similarly, it was observed that those who had delivered in the health centre would immunise their children properly and timely as compared to the mothers who had home deliveries (Table 4).

TABLE 4
FACTORS INFLUENCING HOME DELIVERIES

Variable	Total	Preference for		p-value
		Home Delivery	Hospital Delivery	
Order of pregnancy				
Primi	179	93	86	<0.05
Second or later	147	101	46	
Mother's education				
At least primary school	103	51	52	<0.05
Uneducated	223	143	80	
Father's education*				
At least primary school	129	68	61	<0.05
Uneducated	191	125	66	
Per capita income				
< Rs. 2500	253	167	86	<0.01
> Rs. 2500	73	27	46	
Ante-natal visits				
< 3 visits	68	59	9	<0.001
> 3 visits	258	135	123	
Tetanus toxoid injections				
< 2	45	43	2	<0.001
2	281	151	130	
Iron and folic acid tablets				
< 100	78	63	15	<0.001
> 100	248	131	117	
Initiation of breast feeding				
< half an hour	167	36	131	<0.001
> half an hour	159	158	1	
Pre-lacteal feeds				
Yes	164	147	15	<0.001
No, exclusive breast feeding	162	47	117	
Immunisation				
Incomplete	39	38	1	<0.001
Complete	287	156	131	

* in 4 instances (6 children) the father had expired

CONCLUSION

The Government of India figures suggest that by 1990, only 40-50 per cent of the traditional birth attendants had been trained as against the goal of 80 per cent recommended in the National Health Policy[14]. In 1992 this figure had gone up to 69.4 per cent. As of December 1997, there were an estimated 5067

untrained dais in the State of Karnataka and 1,16,592 untrained dais all over India.

Women in the study area preferred home deliveries as compared to institutional deliveries for various reasons. The cost was the major factor which prevented them from going to the hospital for delivery. Mother's education had a little influence on hospital delivery as compared to father's education. The families with a per capita income of less than Rs.2500/- preferred home deliveries. The reasons offered by the women to prefer hospital delivery were problems during labour (33.3 %) followed by always utilised hospital services (27.3%) and primi gravida (17.4%). The other reasons given by the women include ANC complications, sick during advanced pregnancy, etc.

The socio-economic status of the majority of the rural population is unlikely to change in the near future and home deliveries would continue to prevail in rural areas. Therefore, the authors suggest that the training of traditional birth attendants be undertaken on priority basis. This will ensure that adequate ante-natal, intra-natal and post-natal care services are available to the numerous mothers who are pregnant in the rural areas of the country.

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