# EVALUATION OF UROCYTOGRAM AS A METHOE OF HORMONAL ASSESSMENT IN PREGNANCY, A COMPARATIVE STUDY OF URINARY AND VAGINAL CYTOLOGY AND CYTOCHEMISTRY

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Assessment of hormonal function in pregnancy is one of the most important methods of diagnosis of pregnancy at term and at various stages. Vaginal cytology has an important place as a simple method for the assessment of hormonal function in pregnancy, but this method has not proved to be very reliable. The evaluation of urocytogram has thrown a new light to solve this problem which has got an immense importance for the obstetrician as well as for would be mothers.

The present study was undertaken with the aim of establishing the normal pattern of urocytogram in different trimesters of pregnancy and at full term, and their comparative value with vaginal smear.

#### Material and Methods

The present study included the estimations of urocytogram and vaginal cytology, glycogen, fats and lipids in urinary cells.

The following groups of cases were taken for study.

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(a) Normal healthy non-pregnant females 20

(i) Follicular phase

(ii) Luteal phase

(b) Normal healthy pregnant females

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- (i) First trimester
- (ii) Second trimester 18
- (iii) Third trimester and at term 15
- (c) In Labour-5

# Preparation and Technique

#### Urocytogram

Twenty c.c. first urine voided in the morning was centrifuged and 5 slides were prepared from the sediment, 2 slides were fixed in equal parts of 95% alcohol and ether for PAS (Pears, 1960) and PAP (Papanicolaou stain) 3 slides were fixed in formaline vapours for 30 minutes for lipids.

(a) Sudan black B Method for Masked lipids Pears (1960).

(b) Oil red O Method for neutral fats (Pears 1960)

(c) Schmorl-s method for lipofucsin

Five glass slides were prepared from the lateral vaginal wall, 2 slides were fixed in equal parts of 95% alcohol and ether for PAS and PAP and 3 slides were fixed in formaline vapours for lipids.

## TABLE I

Shoros the	Variations in India	ces of Menstrual	Cycle (Follicular	and Luteal
	Phase) in Va	iginal Smear and	Urocytogram	

			and the formation of the second s
S. No.	Various îndices	V/u follicular phase	V/u luteal phase
1.	M 1 * SD	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	$\begin{array}{cccccccccccccccccccccccccccccccccccc$
	* SD	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	0 71 29
2.	E1 *	72 ±12.8	28 ± 5.8
		48 ± 6.2	33 activities ± 4.3
3.	K 1 * SD	68 ±11.6	24 ± 3.2
	SD	50 ± 6.8	27 ± 3.1
4.	E 1 * SD	8 ± 0.8	64 ± 5.5
	SD	12 ± 1.4	43 ± 3.9
5.	CC 1 * SD	20 ± 2.4	60 ± 7.8
	SD	24 ± 3.4	40 ± 4.1
6.	NC * SD	10 ± 3.2	22 ± 3.4
	SD	8 ± 1.2	16 ± 2.2

\* All difference significant at P 1% is vaginal and urinary counts.

S.D. denotes studard deviation.

## Discussion

The study of urocytogram in present series in follicular phase shows superficial cells with a mean value of  $55\% \pm$ SD 6.2, E 1 with a mean value of  $48\% \pm$  $\pm$  SD 6.2, K 1 with a mean value of  $50\% \pm$  $\pm$  SD 6.8, F 1 with a mean value of  $12\% \pm$  $\pm$  SD 1.4, CC1 with a mean value of  $24\% \pm$  $\pm$  SD 3.4, navicular cells with a mean value of  $80\% \pm$  SD 1.2. The same findings are there in vaginal cytology, that is the increase in mean percentage of superficial cells, K1 and E1, while there is a fall in the F1, CC1 and navicular cells.

Tyler in 1964 has reported that superficial cells are seen in increased numbers, that the maximum cornification is present in follicular phase and leucocytes are decreased. These findings are comparable with the findings of the present study. TABLE II

Shows the various indices in different trimesters of pregnancy and labour in vaginal smear and urocytogram

Sl. no.	Various indices	1st trimester	2nd trimester	3rd trimester	Labour
1.	M1 SD	0:84:16	0:90:10	0:94:6	0:94:6
	SD	$\pm 01 \pm 4.6 \pm 1.2$ 0:76:24	$\pm 0.2 \pm 5.2 \pm 1.3$ 0:86:14	$\pm 0.2 \pm 6.2 \pm 1.1$ 0:96:4	$\pm 2 \pm 6.2 \pm 1.1$ 0:91:9
		$\pm 0.2 \pm 8.4 \pm 3.2$	$\pm 8.9 \pm 2.1$	±11.2 ±0.5	±8.4 ±1.2
2.	E1 SD	16	14	14	:6
		± 2.4	± 1.4	± 1.2	± 0.8
	SD	20	17	10	9
	4	± 2.4	± 2.6	± 2.0	± 1.2
3.	K1 SD	14	10	. 6	6
		± 1.3	$\pm$ 1.1	± 2.0	± 2.0
	SD	20	14	10	8
		± 2.0	± 2.7	± 2.0	± 2.8
4.	F1 SD	84	86	90	92
	"	± 8.6	± 8.4	± 9.8	±10
	SD	43	82	. 85	85
		± 8.4	± 4.1	± 8.5	± 9.8
5.	CC1 SD	70	72	74	72
		± 6.5	± 7.2	± 7.6	± 9.8
	SD	66	67	70	70
		± 5.1	± 4.8	± 6.8	± 9.8
6-	NC SD	44	52	76	75
		± 5.1	± 4.7	± 6.8	± 6.8
	SD	34	40	75	70
		± 4.1	± 3,2	± 8.8	± 6.8

No difference in the three trimesters was significant. Above table shows that practically there is no difference in different i cices between 3rd trimester and labour.

TABLE III

Shows a comparison of mean percentage of mask lipids in normal healthy pregnant and healthy non-pregnant females in urinary cells

	Normal pregnant cases			Non-pregnant cases			
	1st Trimester	2nd trimester	3rd trimester	Follicular phase	Luteal phase		
% of cells with coarse granules	48** ±5.7	52** ±6.8	68** ±5.9	6 ± 0.9	40* ±5.2		
± of cells with fine granules	3 ±0.5	3 ±0.3	2 ±0.4	31 ± 2.8	7* ±0.9		
Urocytogram	0:76 ±02±8.4	24:0:86: ±3.2 ±8.9	140:94.4 ±2.1±11.2	$0:45:55 \pm 0.5 \pm 4.5 \pm 6.2$	0:71:29 ±6.8 ±3.		

\* Difference from follicular phase significant at P1%

\* Difference from luteal phase significant at P = 1%

TABLE IV Shows a comparison of percentage of lipofuchsin in urinary cells in normal preg- nant and non-pregnant females with urccytogram	Normal pregnancy Non-pregnant	1st trimester 2nd trimester 3rd trimester Follicular Luteal phase phase	tive 14 <sup>6</sup> 30 <sup>6</sup> 42 <sup>6</sup> Neg. 30 <sup>6</sup> ± 5.8 ± 5.2 ± 0.8	$0:76:34$ $0:96:14$ $0:96:40$ $45:55$ $0.71$ $29$ $\pm 0.2 \pm 8.4 \pm 3.2$ $\pm 8.9 \pm 21$ $\pm 11.2 \pm 0.2$ $\pm 0.5 \pm 4.5 \pm 6.2$ $\pm 6.8 \pm 3.2$	* Difference from follicular phase significant at P1%	TABLE V Shows a comparison of neutral fat in normal non-pregnant and normal healthy pregnant females	Normal pregnant Non-pregnant	1st trimester 2nd trimester 3rd trimester Follicular Luteal	itive Neg Neg Neg Neg Neg	0:76:24 0:86:14 0:96:2 0:45:55 0:71:29 ±0.1 ±8.4 ±3.2 ±8.9 ± 2.1 ±11.2 ±0.5 ± 4.5 ± 6.2 ± 6.8 ± 3.2	
Shows			% of cells positive	Urocytogram	* Difference from follic	Show			% of cells positive	Urocytogram	

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at

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significant

Difference

: :

Difference significant from luteal phase P

3.2 H Luteal ± 3.4 phase 0:71:29 30\* 6.8 Non-pregnant +1 Shows a PAS material comparison of mean percentage of PAP material in urinary cells in normal pregnant and healthy non-pregnant females with wrocytogram 6.2 Follicular H # 2.0 phase 0:45:55 31 4.5 H 0.5 trimester + 8.9 +1 0:96:4 16\*\*\*  $\pm 11.2$ 3rd Normal pregnancy 2.1 2nd trimester 0:86:14 +1 1% H 6.3 ++69 11 8.9 4 +1 at from follicular phase  $\pm 0.2 \pm 8.4 \pm 3.2$ trimester 0:76:24 + 7.2 lst \* Difference significant of cell positive Urocytogram 20

TABLE VI

In luteal phase the findings of the present series are increase in mean percentage of intermediate cells  $71\% \pm SD$ 6.8, F1  $43\% \pm SD$  3.9, CC1 40% $\pm$  SD 4.1 and navicular cells 18% $\pm 2.2$ , while fall in the mean percentage of E1  $33\% \pm SD$  4.3, K1  $27\% \pm 3.1$ . The same findings are of vaginal cytology. All the differences in all indices are significant in vaginal and urinary counts.

Tayler in 1964 has reported decrease in cornified cells and increase in erythrocytes, basophilic cells and navicular cells. These findings are similar to that of the present series.

Urocytogram during pregnancy in all the trimesters in the present series shows increase in mean percentage of intermediate cells 76% ± SD 8.4, 86% ± SD 8.9 and 96%  $\pm$  SD 11.2, respectively in 1st, 2nd, 3rd trimesters and shows a fall in the mean percentage of E1 and K1 from 20%  $\pm$  SD 2.4 to 17%  $\pm$  SD 2.6 and 10% ± SD 2.0 from 1st to 3rd trimester. The navicular cells are maximum in the 3rd trimester or at term, that is mean value of navicular cells  $34\% \pm$ SD 4.1 in the first trimester and 75%  $\pm$  SD 8.8 in the 3rd trimester. The findings are more or less same in vaginal cytology. No difference in all the three trimesters is significant statistically.

Lencioni in 1969 has reported low value in eosinophilic and karyopyknetic indices. He further reports that smear mainly consists of large number of intermediate cynophilic cells and very few parabasal cynophilic cells upto 37th week of pregnancy. Except parabasal cells findings of Lancioni, the findings of the present series are the same.

Lencioni (1969) has further reported that the superficial eosinophilic cells are 1% upto 37 week of pregnancy, while in this series it is  $4\% \pm$  SD 0.5. The eosinophilic superficial cells are 5% upto 20th week of pregnancy in Lencioni series, while in this series it is with a mean value of  $14\% \pm SD 2.4$ . Parabasal cynophilic cells are less than 1% upto 20th week whereas from 20th to 27th week, 99% of the cases showed 1% or less, while in the present series the parabasal cells are absent.

Lencioni et al (1969) have also reported the increased desquamation cellular clustering folding, and navicular cells between the 20th and 37th weeks of pregnancy. The findings of the present study are compatible with the findings of Lencioni.

The percentage of E1 in urocytogram in the present series is with a mean value of  $4\% \pm SD$  0.5 during the last three weeks of pregnancy. In this case the findings of this series are different from that of Lencioni.

In the present series there is no difference between the findings at the time of labour and at term in urocytogram. The general pattern of the smear in labour is similar to that which is seen at the end of pregnancy.

The findings of the urocytogram corresponds with the findings noted by Pundel in Van Meensal in vaginal smears consisting of low value for superficial eosinophilic cells throughout pregnancy. There is no change in the percentage of cells towards the end of pregnancy and labour, in spite of the progressive increase in the quantity of oestrogen and progesterone.

#### Lipids

The finding of the present study of mask lipids in urinary cells show an increase in coarse lipid granules in luteal phase with a mean value of  $40\% \pm SD$  5.2 as compared to follicular phase  $6\% \pm SD$  0.9 and fall in fine lipid granules in luteal phase with a mean value of  $7\% \pm$ SD 0.9 as compared to  $31\% \pm 2.8$  in follicular phase. The percentage of coarse lipid granules also show an increase in all the three trimesters with a mean value of  $48\% \pm$  SD 5.7,  $52\% \pm$  SD 6.8, and  $68\% \pm 5.9$ , respectively compared to 40% $\pm$  SD 5.2 of luteal phase. All these differences are significant statistically from the luteal phase.

The study of lipofucsin in the urinary cells in the present study shows an increase in all the conditions with progesterone effect in the luteal phase and pregnancy. It was negative in the follicular phase. The increase from 1st to 3rd trimester was interesting, even the difference between luteal phase and trimester was significant.

The study of PAS material in urinary cells in healthy non-pregnant females show an increase of positive cells in luteal phase and in all the trimesters as compared to follicular phase. The mean value of percentage of PAS positive cells in urine is  $12\% \pm \text{SD} 2.0$  in follicular phase, luteal phase  $30\% \pm \text{SD} 3.4$  1st trimester,  $60\% \pm \text{SD} 7.2$  IInd trimester,  $69\% \pm \text{SD} 6.5$  3rd trimester  $76\% \pm \text{SD}$ 8.9. The differences are significant statistically from the follicular phase.

The difference between the luteal phase and 1st trimester may produce a possibility of help in early diagnosis of pregnancy.

The facts brought about by this study are that the urocytogram shows cell patterns parallel to those reflected in the vaginal smears under the influence of the oestrogen and progesterone and the results in various groups give the same information. This has the great advantage that in situations where the vaginal cytology may fail to give results due to inflammation or bleeding the urocytogram may be used as an effective and reliable replacement.

The stuly of lipids and lipofucsins and the polysaccharides (PAS positive material) are also interesting. The phase alteration in the type of masked lipids as observed from follicular to luteal phase and pregnancy reveals that lipids are not static as often presumed but very active participants of cell metabolism. The count of cells with the types of lipid globules may be helpful in early diagnosis of pregnancy.

## Conclusion

1. In normal healthy non-pregnant females the various indices in urocytogram are significant statistically in urinary counts and the findings of the vaginal cytology are parallel to urocytogram.

2. No difference in the three trimester is significant statistically in normal healthy pregnant females.

3. No difference is being noted in between 3rd trimester and labour.

4. The percentage of coarse lipid granules in urocytogram  $40\% \pm 5.2$  in luteal phase is significant statistically than the follicular phase  $6\% \pm 0.9$ .

5. The percentage of coarse lipid granules are significant statistically in all the three trimesters than the luteal phase.

6. The study of lipofucsin in the urinary cells in the present study shows an increase in all the condition with progesteron effect in luteal phase and pregnancy. It was negative in follicular phase. As these may help in early diagnosis of pregnancy.

7. The findings of the present study of

neutral fats in urinary cells has not shown any significance. Hence the further studies are being needed in this field.

8. The difference in positive PAS material are significant statistically in luteal phase with a mean value of  $30\% \pm$  SD 3.4 than in the follicular phase 12%  $\pm$  SD 2.0.

9. The difference in the percentage of positive PAS material in all the trimesters of normal pregnancy are significant than in the luteal phase.

10. The difference between the luteal phase and 1st trimester may produce a possibility of help in early diagnosis of pregnancy.

11. This study of urocytogram shows cells pattern parallel to those reflected in the vaginal smear under the influence of oestrogen and progesterone.

#### References

- Castellanos, H. N. and Stergis, S. H.: Urinary cytology in Endocrine evaluation of normal female Prog. in Gynec. to be published
- Donald, F.: Am. J. of Obst. & Gynec., 90: 149, 1964.
- Maccallin, P. F.: A study of the changes in the cytology of the urinary sediment during pregnancy. Thesis for M.S. in Physiology and Pharmacology University of Colorado 1949.
- Pears, A. G.: Everson Histochemistry, Theoritical and applied Sec. ed. 1960 J & A Churchill pp. 854-925.
- Pundel, J. P. et and Van Meensel, F.: Gestation et cytologic vaginale Masson et cie Paris 1951.
- Roszel, F. Jeffic and Lca G. Back: Acta cytologica. 16: 438, 1972.
- Young, S., Bulbook, R. D. N. and Greenwood, F. C.: Lancet 1: 350, 1957.