Relationship of Maximum Follicular Size, Age of Woman, and Reproductive Implications in Women Attending Fertility Clinic in St. Margaret's Hospital, Lokoja, Using Creighton Model FertilityCare™ System and NaProTECHNOLOGY

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Abstract

Background: Some authorities have found that, when the mean follicular diameter is >25 mm, the follicle is considered large, and these authorities are convinced that large follicles are biomarkers to ovarian pathology. Several studies have been carried out to determine the optimal maximum follicular size (MFS) that will be adequate before the use of human chorionic gonadotropin trigger to induce ovulation. None of these studies have considered the woman's age as a factor. An *in vitro* fertilization-based study has also shown that large leading follicles did not result in higher percentage of matured oocytes, and they even result in a lower live birth rate; however, we note that the ages of the women were not specifically mentioned in connection with the problems of large follicle. **Subjects and Methods:** We set out in this study to evaluate the relationship between the MFS and the woman's age both in our control group (fertile women) Group (A) and in the women attending fertility clinic Group (B). Using the Creighton Model Fertility*Care* System and NaProTECHNOLOGY, it was possible to determine when to start follicular tracking and also determine ultrasound diagnosis of ovulation by recording the MFS and the reduced size. **Results:** In both Group A and Group B, we discovered that the MFS reduces as woman's age increases. We also discovered that the average MFS in the Group B was higher for age compared with the Group A ($P = 0.0043$). There was also an association between these bigger follicles for age and low mid-luteal progesterone. This is probably the first study that describes the association of follicular size and woman's age and its possible link with ovulation defects. **Recommendation:** We have proposed that this phenomenon of large matured maximum follicle for age could be a contributory factor to infertility and miscarriages. We propose further studies to verify this hypothesis. **Conclusion:** Despite the high percentage of complete rupturing of follicles in the AB cycles in the Group (B) there are very high proportion having low mid luteal (P+7) Progesterone. This may be due to pathologies associated with big follicles for woman's age. The E2/Pg ratio study in luteal phase strongly suggested that the women in Group (B) that may have fertility challenge are those in subgroup of AB in Group (B). Therefore matured follicular size should be considered with the woman's age. If the follicles grows bigger away from the range for a woman's age it may be the marker for infertility or reproductive health challenges.

Keywords: Creighton Model FertilityCare™ system, infertility, maximum follicular size, NaProTECHNOLOGY, ovulation, woman's age

Introduction

Professor Hilgers, although did not specifically mention the correlation of "follicular size for age," reported a case of a 30-year-old woman, gravida 0 and para 0, who during ultrasound tracking of follicular growth got a maximum follicular size (MFS) of 31.4 mm, and he judged such follicle to be too large and unhealthy; he said that the average-size follicle prior to rupture (mean follicular diameter [MFD]) is 22 mm. When the MFD is >25 mm, the follicle is considered large, and he proposes that large follicles may be biomarkers to ovarian pathology.[1] In a study by Ojengbede *et al*., normal volunteers had MFS at an average of 20.8 mm (15.5–27.0) mm,

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and this was not significantly different from the infertile women with MFS at an average of 21.4 mm (15–28) mm;[2] we note that the ages of these women were not considered specifically. In an intrauterine insemination study by Palatnik *et al*., studying the optimal follicular size before human chorionic gonadotropin (HCG) trigger in clomid and letrozole treatment cycles and pregnancy outcomes, the authors concluded that the higher pregnancy rates were achieved when the leading follicles were in the range of 23–28 mm;[3] they did not reveal the maternal ages in which those MFSs were attained and pregnancy was achieved. A significant reduction in follicle density and percentage of morphologically normal follicles was detected with increasing age. Reduced fertility with maternal aging has been well documented in humans.[4] We then believe that conducting a study to specifically look into the relationship of MFS prior to ovulation in relation to woman's age both in fertile women and women attending fertility hospital will be a good means of understanding the dynamism of ovulation with age. In NaProTECHNOLOGY (NPT), the mid-luteal progesterone is accurately done to confirm the ovulation by checking the Peak + 7 estrogen and progesterone levels (accurate mid-luteal progesterone assessment).[5] Some studies have shown that increased aromatase activity is associated with increasing follicular growth beyond the antral stage. The largest follicle has the highest intrafollicular levels of estrogen due to its high capacity of androgen aromatization.[6,7] Estrogen has mitogenic and growth-stimulating effects on matured follicles under the influence of follicle-stimulating hormone (FSH).[8] The aim of our study is to find out what relationship the MFS before ovulation has with woman's age, comparing our normal controls with women attending fertility Clinic and also comparing their hormonal values with the Omaha standard values.

Subjects and Methods

Creighton Model FertilityCare™ System (CrMS) and NPT are simple technologies that can be used to assess female fertility. They were used in the evaluation of relationship between MFS and age of woman, comparing controls and those attending Fertility Clinic in St. Margaret's Hospital Lokoja from 2014 to 2017; the results are interpreted on the background of standard Creighton Model chart.

Subjects

Group A (control)

Group A constituted women with normal fertility. Their hormonal profile was compared with normal fertility study data of 57 women studied at Pope Paul VI Institute for the Study of Human Reproduction [Tables 3a, b and 5a-d]. They were all regularly ovulating. A total of nine women were recruited and 15 cycles were studied. Their age ranged from 23 to 45 years. They were taught how to chart the CrMS and they were all charting the CrMS fertility chart at the time of study. The participants gave verbal consent to the study. There was no financial inducement.

Group B (women attending fertility clinic)

These women were recruited into the study because they presented to be evaluated for fertility. Note that these women were not classified as being infertile. The criterion that qualified them to be in Group B was that they presented for fertility evaluation. They all desired pregnancy. They were taught how to chart the CrMS and they were all charting the CrMS fertility chart at the time of study. One of the women became pregnant during the evaluation cycle. Thirty-seven women were recruited and 37 cycles were studied. Their age ranged from 25 to 46 years. Their hormonal profile was compared with normal fertility study data of 57 women studied at Pope Paul VI Institute for the Study of Human Reproduction [Tables 4a-b and 5a-d]. All the women signed the CrMS consent form which allows their data to be used in research studies. There was no financial inducement.

Ethical clearance certificate was provided by the Director of Medical Services and Training, Kogi State Ministry of Health, after the proposal of the study was sent in for approval.

The methods used to evaluate the women in Group A and Group B included the following:

- Analysis of the charting pattern of the CrMS after they have been taught and developed confident identification of peak day. We paid attention to the biomarkers, namely cycle length, prepeak phase, postpeak phase, and peak day
- Targeted hormonal assay: periovulatory estrogen to identify E-2, PE2, and E + 2 and postovulatory estrogen and progesterone at $P + 3$, $P + 5$, $P + 7$, $P + 9$, and $P + 11$, [Figures 1-4 and Tables 3a, b, 4a-b, 5a]
- Targeted serial ultrasound scanning: Transabdominal starting from $P - 6$, $P - 4$, $P - 2$, P, and $P + 2$ to identify the dominant follicle and follow it until it ruptures. The MFS and the reduced follicular size (RFS) were calculated by (L + B + T)/3 = MFD [Tables 1a and 2a]
- In the control Group (A), we plotted a graph of the MFS (Y-axis) against the age of the women (X-axis) and connected the highest follicular size attained by the youngest woman to the highest follicular size attained by the oldest woman. We now used the graph developed as our standard to compare with the women in Group (B) that presented for fertility evaluation [Figure 5]
- AB = Cycle in which the MFS was matured but above the graph drawn line for age in the control
- BL = Cycle in which the MFS was matured but below the graph drawn line for age in the control
- OL = Cycle in which the MFS was matured and on the graph line drawn for age in the control.

Creighton Model FertilityCare System and NaProTECHNOLOGY

Figure 6a shows a typical Creighton Model chart. This chart is developed by the women after they have attended a fertility class (introductory session [IS]), conducted by a well-trained

Table 1a: Normal fertility control Group (A)

Cycle no	LMP	MFS mm	RFS/FRD mm	DMFS	DRFS	PD	POD	AGE	ESTROGEN LEVEL ON DMFS Pg/ml			PPP	P+7		RATIOE2/ Pg	Zone
									E-2	PE2	E+2		E2Pg/ ml	PGng/ ml		
1	05/05/14	21.7	9.1/(CR)	13	15	17	15	23		328		10	76	17.8	4.3	BL
2	22/11/14	18.8	11.6/(PRS)	12	14	15	14	23		530		13	300	19.5	15.4	BL
3	04/11/14	24.9	9.2/(CR)	16	18	20	18	23		335		10	220	31	7.1	OL
4	08/08/14	19.2	3.6/(CR)	11	13	13	13	38		260		14	380	27	14.1	BL
5	17/12/14	19.9	13.4/(PRS)	10	12	13	12	38		1100		14	650	14	46.4	BL
6	29/01/17	22.7	10.8/(CR)	13	15	13	15	26		300		12	305	55	5.5	BL
7	10/05/17	20.1	13.2/(PRS)	13	15	13	15	26		225		13	115	17.5	6.6	BL
8	29/06/17	20.8	14.1/(PRS)	14	16	16	16	26		220		11	130	12.5	10.4	BL
9	26/07/17	20.8	10.9/(CR)	13	15	14	15	26		275		11	298	31	9.6	BL
10	18/03/17	19.4	9.7/(CR)	15	17	16	17	25		597		12	375	32.5	11.5	BL
11	02/11/17	20.2	No follicle seen/(CR)	13	15	15	15	30		205		14	150	25	6.0	BL
12	22/11/16	21.4	14/(PRS)	19	21	18	21	26		438		12	228	15	15.2	BL
13	20/11/17	19.3	11.6/(CR)	10	12	12	12	41		205		14	158	23.1	6.8	BL
14	11/12/17	20.3	10.0/(CR)	10	12	11	12	45		275		16	103	28.5	3.6	OL
15	16/12/17	19.0	10.4/(CR)	12	14	14	14	41		225		16	170	26.5	6.4	BL

LMP: Last mestrual period. PD: Peak day; PPP: Post peak phase, MFS: Maximum follicular size, POD: Predictive ovulation day, RFS: Reduced follicular size, E-2: Estrogen Level 2 Days Before PE_2, DMFS: Day of maximum follicular size, PE_2: Peak estrogen level in follicular phase, DRFS: Day of reduced follicular size, E+2=Estrogen Level 2 Days After PE_2, AB: Matured follicle grown above maximum control line for age, BL: Matured follicle grown below maximum control line for age, OL: Matured follicle grown on the maximum control line for age

Table 1b: Normal fertility control Group (A) showing estrogen sum (3 value)

Cycle no	LMP	Age years	ESTROGEN LEVEL ON DMFS Pg/ml			E2 SUM (3 value) (424-648) pg/ml	P+7		Ratio	Zone
			E-2	PE2	E+2		E2 Pg/ml (73-167)	PG ng/ml (10-21.4)		
1	05/05/14	23	68	328	112	508	76	17.8	4.3	BL
2	22/11/14	23	295	530	305	1130	300	19.5	15.4	BL
3	04/11/14	23	265	335	85	685	220	31	7.1	OL
4	08/08/14	38	140	260	60	460	380	27	14.1	BL
5	17/12/14	38	165	1100	750	2015	650	14	46.4	BL
6	29/01/17	26	163	300	158	621	305	55	5.5	BL
7	10/05/17	26	130	225	90	445	115	17.5	6.6	BL
8	29/06/17	26	128	220	100	448	130	12.5	10.4	BL
9	26/07/17	26	128	275	83	486	298	31	9.6	BL
10	18/03/17	25	305	597	300	1202	375	32.5	11.5	BL
11	02/01/17	30	130	205	125	460	150	25	6.0	BL
12	22/11/16	26	303	438	175	916	228	15	15.2	BL
13	20/11/17	41	150	205	120	475	158	23.1	6.8	BL
14	11/12/17	45	200	275	108	583	103	28.5	3.6	OL
15	16/12/17	41	175	225	138	538	170	26.5	6.4	BL

LMP: Last mestrual period. E-2: Estrogen level 2 days Before PE_2, PE_2: Peak estrogen level in follicular phase, E+2: Estrogen level 2 days after PE_2, AB: Matured follicle grown above maximum control line for age, BL: Matured follicle grown below maximum control line for age, OL: Matured follicle grown on the maximum control line for age

fertility care practitioner (FCP). This IS lasts between 4 and 6 hours depending on the size of each class and the various questions that arise from the class. Almost all women >98% will be able to start immediately to observe their various biological markers (biomarkers) and represent them on their chart from the very 1st day of attending the "IS." The biomarkers are observed at the vaginal outlet using flat toilet tissues to wipe the vaginal outlet from the urethra, through the vagina to the perineal body (front to back). The times to make observations included at urination, at defecation, and at bathing before and after. There is also the last observation to be made at the end of the day when the woman bears down gently and do the final wiping. These observations are very easy and the FCP will give the necessary motivation, while the

Table 2a: Women attending fertility clinic Group (B)

Cycle no	LMP	MFS mm	RFS/FRD mm	DMFS	DRFS	PD	POD	Age years	ESTROGEN LEVEL ON DMFS Pg/ml			PPP	P+7		Ratio	Zone
									E-2	PE2	E+2		E2 Pg/ml	PG ng/ml		
1	14/09/16	21.93	11.9/(CR)	12	14	15	14	25		250		Pregnant	213	12.0	17.8	BL
2	30/12/16	23.93	14.4/(CR)	11	13	15	13	36		355		12	425	9.0	47.2	AB
3	17/07/15	23.93	16.5/(PRS)	12	14	14	14	39		550		15	600	8.5	70.6	AB
4	23/04/15	20.23	11.3/(CR)	9	11	-	11	31		300		-	362.5	16.2	22.4	BL
5	08/09/15	23.0	No follicle seen/(CR)	13	15	15	15	36	213			11	138	4.0	34.5	AB
6A, 6B	28/10/15	21.0; 23.3	No follicle seen/(CR)	11	12	13	12	33		275		15	275	3.9	70.5	BL OL
7	13/09/15	25.8	9.5/(CR)	13	15	16	15	32		1000		10	150	5.0	30.0	AB
8	17/08/16	28.0	23.0/(PRS)	12	14	14	14	33			550	13	625	18.0	34.7	AB
9	09/06/16	28.1	10.0/(CR)	11	13	11	13	33		950		14	250	6.0	41.7	AB
10	18/06/16	21.9	16.8/(PRS)	14	16	17	16	33			525	10	525	6.5	80.8	BL
11	10/05/16	20.8	19.7/(PRS)	14	16	18	16	41		150		10	100	2.0	50.0	BL
12	01/03/16	22.5	9.8/(CR)	12	14	15	14	30	250			14	225	10.0	22.5	BL
13	26/01/17	21.1	No follicle seen/(CR)	15	17	15	17	35		602		14	225	5.3	42.5	BL
14	29/12/16	20.0	11.2/(CR)	13	15	14	15	32		238		16	125	7.0	17.9	BL
15	24/12/15	24.0	No follicle seen (CR)	12	14	14	14	26		375		Pregnant	125	16.0	7.8	BL
16	14/01/16	22.5	14.5/(CR)	16	17	15	17	30		200		15	275	7.5	36.7	BL
17	01/07/17	21.2	13.7/(CR)	13	15	16	15	30		275		12	170	31.0	5.5	BL
18	25/05/15	19.0	13.2/(PRS)	10	12	12	12	32	238			15	195	7.0	27.9	BL
19	09/06/16	24.3	13.1/(CR)	17	19	15	19	40		350		13	263	17.0	15.5	AB
20	20/06/15	21.1	No follicle seen/(CR)	14	16	18	16	40		850		12	63	16.0	3.9	BL
21	25/10/15	20.0	12.7/(PRS)	12	14	15	14	29		566		11	150	11.1	13.6	BL
22	10/05/17	22.1	11.0/(CR)	10	12	12	12	39		298		14	400	14.0	28.6	AB
23	18/09/16	18.0	11.0/(PRS)	10	12	-	12	46		750		-	225	6.5	34.6	BL
24	02/01/16	22.7	12.3/(CR)	14	16	15	16	37		225		12	70	6.6	10.6	AB
25	06/04/16	22.4	11.4/(CR)	13	15	14	15	32			62.5	13	387.5	1.2	322.9	BL
26	11/10/15	31.1	6.5/(CR)	10	12	11	12	28		525		13	113	20.0	5.65	AB
27	10/06/15	24.0	12.0/(CR)	10	13	14	13	29		525		12	250	8.0	31.3	AB
28	08/04/15	18.9	11.95/ (PRS)	12	14	14	14	39		312.5		12	500	6.0	83.3	BL
29	09/10/15	24.1	7.7/(CR)	10	12	13	12	31		1000		11	375	3.6	104.2	AB
30	19/12/16	23.0	No follicle seen/(CR)	15	17	16	17	26		275		14	175	13.5	12.9	BL
31	12/05/17	24.1	14/(CR)	15	16	13	16	34			337.5	16	175	17.5	10.0	AB
32	29/05/17	19.0	8.7/(CR)	14	16	13	16	31	225			16	153	18.0	8.5	BL
33	27/05/17	18.9	3.0/(CR)	10	12	13	12	34	134			14	172	16.0	10.8	BL
34	14/03/17	17.7	No follicle seen/(CR)	12	14	14	14	41		1100		15	700	20.5	34.1	BL
35	05/06/17	16.9	No follicle seen/(CR)	18	20	17	20	38		229		12	106	16.5	6.4	BL
36	15/10/15	30.0	19.4/(CR)	14	16	17	16	29		775		14	320	5.8	55.2	AB
37	21/12/16	20.9	13.2/(CR)	12	14	15	14	37		250		15	155	1.1	140.9	BL

LMP: Last mestrual period. PD: Peak day; PPP: Post peak phase; CR: Complete rupture (MFS-RFS >/= 7.5), MFS: Maximum follicular size, POD: Predictive ovulation day; PRS: Partial rupture syndrome (MFR-RFS <7.5), RFS: Reduced Follicular Size, E-2: Estrogen level 2 days Before PE_2, DMFS: Day of maximum follicular size, PE_2: Peak estrogen level in follicular phase; FRD: Follicular rupture difference (MFS-RFS), DRFS: Day of reduced follicular size, E+2: Estrogen Level 2 Days After PE_2, AB: Matured follicle grown above maximum control line for age, BL: Matured follicle grown below maximum control line for age, Ol: Matured follicle grown on the maximum control line for age

husbands support their wives. Various stamps with different color codes are given to the women and they use the stamps accordingly based on what they observe on a daily basis. The "RED" stamp is to mark days of bleeding, which will include

Table 2b: Women attending fertility clinic Group (B) showing estrogen sum (3 value)

CYCLE NO	LMP	AGE Years	ESTROGEN LEVEL ON DMFS Pg/ml			E_2 SUM (3 value) (424-648) pg/ml	P+7		RATIO	ZONE
			E-2	PE_2	E+2		E_2 Pg/ml	PG ng/ml		
1	14/09/16	25	175	**250**	125	550	213	12	17.8	BL
2	30/12/16	36	200	**355**	170	725	425	9	47.2	AB
3	17/07/15	39	325	**550**	225	1100	600	8.5	70.6	AB
4	23/04/15	31	175	**300**	188	663	363	16.2	22.4	BL
5	08/09/15	36	**213**	300	250	763	138	4	34.5	AB
6A	28/10/15	33	188	**275**	120	583	275	3.9	70.5	BL
6B	28/10/15	33	188	**275**	120	583	275	3.9	70.5	OL
7	13/09/15	32	650	**1000**	250	1900	150	5	30.0	AB
8	17/08/16	33	575	625	**550**	1750	625	18	34.7	AB
9	09/06/16	33	230	**950**	208	1388	250	6	41.7	AB
10	18/06/16	33	275	1000	**525**	1800	525	6.5	80.8	BL
11	10/05/16	41	100	**150**	73	323	100	2	50.0	BL
12	01/03/16	30	**250**	1000	138	1388	225	10	22.5	BL
13	26/01/17	35	318	**602**	116	1036	225	5.3	42.5	BL
14	29/12/16	32	155	**238**	200	593	125	7	17.9	BL
15	24/12/15	26	150	**375**	110	635	125	16	7.8	BL
16	14/01/16	30	85	200	**188**	473	275	7.5	36.7	BL
17	01/07/17	30	205	**275**	73	553	170	31	5.5	BL
18	25/05/15	32	**238**	650	275	1163	195	7.0	27.9	BL
19	09/06/16	40	328	**350**	250	928	263	17	15.5	AB
20	20/06/15	40	475	**850**	275	1600	63	16	3.9	BL
21	25/10/15	29	55	**566**	288	909	150	11	13,6	BL
22	10/05/17	39	138	**298**	49	485	400	14	28.6	AB
23	18/09/16	46	200	**750**	213	1163	225	6.5	34.6	BL
24	02/01/16	37	128	**225**	50	403	70	6.6	10.6	AB
25	06/04/15	32	187.5	275	**62.5**	525	387.5	1.2	322.9	BL
26	11/10/15	28	288	**525**	200	1013	113	20	5.65	AB
27	10/06/15	29	250	**525**	125	900	250	8	31.3	AB
28	08/04/15	39	250	**312.5**	100	662.5	500	6	83.3	BL
29	09/10/15	31	325	**1000**	175	1500	375	3.6	104.2	AB
30	19/12/16	26	100	275	202	577	175	13.5	12.9	BL
31	12/05/17	34	500	825	337.5	1662.5	175	17.5	10.0	AB
32	29/05/17	31	225	400	130	755	153	18	8.5	BL
33	27/05/17	34	**134**	178	70	382	172	16	10.8	BL
34	14/03/17	41	450	**1100**	325	1875	700	20.5	34.1	BL
35	05/06/17	38	89	229	151	469	106	16.5	6.4	BL
36	15/10/15	29	201	775	78	1054	320	5.8	55.2	AB
37	21/12/16	37	25	250	124	399	155	1.1	140.9	BL

LMP: Last mestrual period. E-2: Estrogen level 2 days before PE_2, PE_2: Peak estrogen level in follicular phase; E+2: Estrogen level 2 days after PE_2, AB: Matured follicle grown above maximum control line for age, BL: Matured follicle grown below maximum control line for age; OL: Matured follicle grown on the maximum control line for age

menstrual flow, or any other bleeding event that may occur. Any day she sees blood MUST have RED stamp irrespective of what day in her cycle it occurred. The numbers 1–35 on the top of the chart represent the days of cycle and not the days of the month. Immediately below the stamp space is the location for writing the date of the month. For example, if a woman starts her menses on December 27, then day 1 on the chart will be dated December 27 and day 2 will be December 28. And immediately below the date space is the space for recording of the observation using the code of the Vaginal Discharge Recording System [Figure 6b].

A well-trained FCP and a NaPro medical consultant can interpret this code to generate a standardized objective evaluation from a subjective vulva observation. Once the menses starts again, she moves down to start a new cycle. The "Plain Green" stamps are used to mark the days the woman experience dryness; for example in Figure 6a, she has dryness from 9th to 13th days of her cycle in the pre-peak phase (follicular phase) and dryness from 20th to 29th days of her cycle in the postpeak phase (luteal phase) of her cycle. In principle, prepeak phase (days 1–19) is from the 1st day of menstrual flow up until including the peak day. While the

Table 3: Distribution of AB, BL, OL, by age ranges

Age range years	GROUP B			Total
	AB	BL	OL	
25-27	-	2	1	3
28-30	3	3	1	7
31-33	6	5	1	12
34-36	3	1	1	5
37-39	4	2	-	6
40-42	3	1	-	4
43-45	-	-	-	-
46-48	1	-	-	1
Total	20	14	4	38

AB=Matured follicle grown above maximum Control line for age.
BL=Matured follicle grown below maximum Control line for age.
OL=Matured follicle grown on maximum Control line for age

postpeak phase (days 20–29) is from the 1st day after the peak day till the last day before the beginning of the next menstrual period. The CrMS study has revealed that the postpeak phase is relatively constant in length (12 days ± 4), whereas the prepeak phase is variable in length. The peak day is defined as the last day of the observation of cervical mucus discharge at the vulva that is clear, stretchy, or lubricative. Each time the woman observes mucus, she has to use the "White Baby" stamps. And, the mucus could be a peak type or nonpeak type. Peak-type mucus is either stretchy in consistency, clear in color, or lubricative in sensation. Any one of these qualities qualifies the mucus as peak type. While the nonpeak-type mucus is the mucus discharge that is not clear, not stretchy, and not lubricative. The "Green Baby" stamps are special stamps used to indicate the period after the peak day when

Table 3a: Luteal phase estrogen (pg/ml) data showing control Group A, and omaha study

	OMAHA	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
P+3	88	284	340	285	92	900	170	70	103	113	225	100	175	-	63	-
P+5	108	300	825	265	220	800	125	168	100	245	250	173	188	-	130	-
P+7	120	76	300	220	380	650	305	115	130	298	375	150	228	158	103	170
P+9	120	48.8	-	525	208	1075	95	220	208	205	550	225	100	-	130	-
P+11	98	56	-	315	96	950	100	305	125	148	225	95	163	-	150	-

Table 3b: Luteal phase progesterone (ng/ml) data showing control Group A and omaha study

	OMAHA	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
P+3	9.4	26.2	18	40	25	24	37	7.5	16	13.5	12	25	1.6	-	14.5	-
P+5	14.4	19.7	10	56	26.5	23	44	22.5	20	37.5	10	22	6.8	-	23	-
P+7	15.7	17.8	19.5	31	27	14	55	17.5	12.5	31	32.5	25	15	23.1	28.5	26.5
P+9	13.6	13	-	12	33	30.5	27	26.5	20.5	38.5	20	12	12	-	26.5	-
P+11	8.1	12.2	-	8	19	12	3	20	7.5	15	22	2	9	-	10	-

Table 4a: Luteal phase estrogen (pg/ml) data showing Group B, and omaha study

	OMAHA	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
P+3	88	175	375	225	200	400	500	250	638	208	325	75	250	488	130	50
P+5	108	100	450	550	250	263	125	-	475	208	438	138	400	249	178	125
P+7	120	213	425	600	362.5	138	175	150	625	375	525	100	100	225	125	125
P+9	120	238	250	675	600	250	300	150	525	250	225	55	88	310	288	175
P+11	98	150	180	275	500	125	175	63	288	250	350	45	50	164	375	175

	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
P+3	150	113	340	250	175	140	353	-	100	175	300	225	312.5	525	178	337.5
P+5	85	128	175	225	25	120	153	-	100	575	200	250	400	600	123	75
P+7	275	170	275	263	63	150	400	225	70	387.5	113	250	500	375	150	175
P+9	175	200	195	190	45	438	375	-	100	387.5	100	150	450	83	120	288
P+11	84	205	275	230	25	100	600	-	50	75	211	75	250	58	120	450

	32	33	34	35	36	37
P+3	400	118	650	-	71	-
P+5	130	136	400	-	125	-
P+7	153	172	700	106	320	155
P+9	145	134	275	-	200	-
P+11	180	106	250	-	125	-

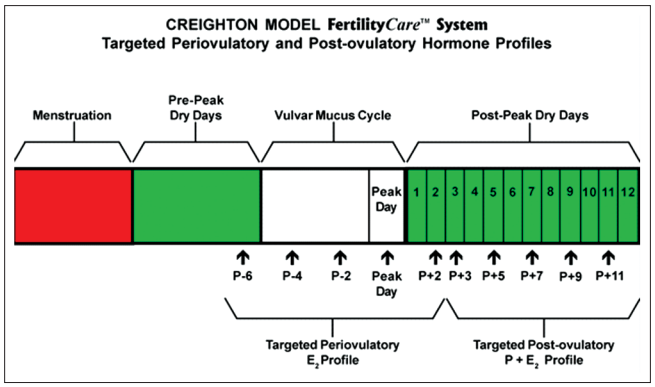

Figure 1: This schematic drawing of the Creighton Model FertilityCare System demonstrates how it is used to target the periovulatory and postovulatory hormone profiles

Mean and Standard Deviation of Post-Peak Progesterone Profile and Progesterone Sum and Mean Normal Controls (N=57)

Day of Profile	Mean (ng/mL)	SD[6]
P+3	9.4	4.1
P+5	14.4	4.7
P+7	15.7	5.7
P+9	13.6	5.3
P+11	8.1	5.0
P Sum (5 values)[4]	61.2	17.4
P Mean (5 values)[5]	12.3	3.4

Figure 3: These normal values apply only to the laboratory methods used at the National Hormone Laboratory of the Pope Paul VI Institute. Obtained from the endocrine evaluation of 57 spontaneous cycles which were sonographically identified as containing a mature follicle with a positive cumulus oophorus and complete rupture (anatomically normal ovulation by ultrasound). From: Hilgers TW: The Medical and Surgical Practice of NaProTechnology. Pope Paul VI Institute Press, Omaha, Nebraska, 2004

Mean and Standard Deviation of Periovulatory Estradiol-17β (E_2) Profile E_2 Sums and Means (3 and 4 Value)—Normal Controls (N=57)

Day of Profile	Mean (ng/dL)	SD
E–4	8.8	2.1
E–2	14.8	3.1
Peak E_2	26.0	5.9
E_2+2	12.8	6.6
E_2 Sum (3 value)	53.6	11.2
E_2 Sum (4 value)	63.2	14.5
E_2 Mean (3 value)	17.9	3.7
E_2 Mean (4 value)	15.8	3.6

Figure 2: These normal values apply only to the laboratory methods used at the National Hormone Laboratory of the Pope Paul VI Institute. The E2 profile is obtained by drawing E2 levels every other day from $P - 5$ (or $P - 6$) through $P + 2$ (with reference to the CREIGHTON MODEL Fertility*Care*TM System), obtained from the endocrine evaluation of 57 spontaneous cycles which were sonographically identified as containing a mature follicle with a positive cumulus oophorus and complete rupture (anatomically normal ovulation by ultrasound). The E2 sum is obtained by adding the E2 values in the E2 profile to include the level before the peak level, the peak level and the level after the peak level (3 value sum) or the two levels before the peak level, the peak level and the level after the peak level (4 value sums). The E2 mean is the average of the 3 (3 value) or 4 (4 value) levels that are used to make up the 3- and 4-value sums. SD = Standard deviation. From: Hilgers TW: The Medical and Surgical Practice of NaProTechnology. Pope Paul VI Institute Press, Omaha, Nebraska, 2004

Day of Profile	Mean (ng/dL)	SD[6]
P+3	8.8	3.5
P+5	10.8	4.4
P+7	12.0	4.7
P+9	12.0	4.7
P+11	9.8	5.7
E_2 Sum (5 values)[4]	53.2	20.2
E_2 Mean (5 values)[5]	10.7	4.0

Figure 4: The postpeak Estrogen from the study of 57 normal ovulating women with evidence of Cumulus oophorus and follicular rupture ≥7.5 mm

the cervix is closing with dryness but still fertile. The NPT study has shown that the cervix closes over 3 days after the peak. The period of ovulation occurs 95.4% from peak −2 to peak +2. Ovulation is always 100% completed at peak ±3.[9] The cervical mucus which the woman observes is a reflection of the response of the cervix to the estrogen which the developing follicles produce. And, the NPT has developed a standardized scoring system for the mucus which women observe.[10,11] The grading of the mucus scores is as follows: regular 9.1–16; intermediate regular 7.6–9.0; intermediate limited 5.7–7.5; limited 0.1–5.6; and dry = 0. To calculate this mucus score, we start from the peak day and count six steps backward. All the mucus observed on these days ($P - 5$), + ($P - 4$), + ($P - 3$), + ($P - 2$), + ($P - 1$), + (peak day) are calculated and the sum is divided by 6. The mucus is scored on four characteristics (3CS), namely: (1) consistency (length of mucus), 6 and 8 are both scored 2. The mucus that is stretchy coded 10 is scored 4. (2) color, mucus that is brown, cloudy, or yellow is scored 2, the mucus that is crystal clear, transparent, is scored 4. (3) change, no change in mucus scored 2, and change in mucus scored 4. (4) sensation, The mucus that feels nonlubricative is scored 0, and the mucus that feels lubricative is scored 4. Any day that mucus was not observed is scored 0. For example 1: 8CL means tacky, cloudy, and lubricative. The score; consistency, 8 scored (2), colour, cloudy is scored (2), Changed to Lubricative scored (4), Sensation is Lubricative scored 4. Total = 2 + 2 + 4 + 4 = 12. Example 2: 10 C means

Table 4b: Luteal phase progesterone (ng/ml) data showing Group B, and omaha study

	OMAHA	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
P+3	9.4	12	14	16	7.0	20	12	7.0	11	7.9	20.2	10	1.0	4.2	1.8	13
P+5	14.4	16.5	13	21	17	8.0	4.5	-	16	13	23.6	9.5	1.0	5.0	2.5	19.5
P+7	15.7	12	9.0	8.5	16.2	4.0	4.0	5.0	18	17	6.5	2.0	1.0	5.0	7.0	16
P+9	13.6	13	2.0	6.0	11	3.8	9.0	4.0	12	9.0	3.5	1.5	2.0	13.4	10	19.5
P+11	8.1	3.0	1.3	3.0	10	2.9	3.8	3.0	3.5	6.0	3.0	0.9	3.0	9.2	17	25.5
	16	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**
P+3	6.8	13.5	7.0	2.0	12	25	10.5	-	6.9	2.2	10	14.5	6.0	7.0	1.8	5.3
P+5	12.7	26	6.0	6.0	17	33	12.5	-	9.5	6.5	14	14	2.2	15	10	11
P+7	7.5	31	9.0	17	16	11	14	6.5	6.6	1.2	20	8.0	6.0	3.6	13.5	17.5
P+9	8.1	20.5	7.0	14	5.0	5.0	15	-	5.5	4.0	6.2	4.0	5.0	2.1	6.0	17
P+11	4.3	10.5	7.0	10	2.0	4.1	20	-	2.3	2.2	2.0	1.8	2.4	1.6	5.0	19
	32	**33**	**34**	**35**	**36**	**37**										
P+3	16	5.5	10	-	6.8	-										
P+5	20	6.9	10	-	10	-										
P+7	18	16	20.5	16.5	5.8	1.1										
P+9	22.5	9.4	15	-	10	-										
P+11	20	5.8	14	-	7.1	-										

Table 5a: Periovulatory estrogen (pg/ml) study of 57 (omaha), 9(normal control A, A (BL), A (OL)), and 37 (Group B, B (BL), B (OL), B (AB) attending fertility clinic)

	OMAHA	CONTROL A	Group B	Group A (BL)	Group A (OL)	Group B (BL)	Group B (OL)	Group B (AB)
E-2	148	183	244.4	174.8	232.5	195.8	188	310.8
PE2	260	361.2	515.6	377.5	305	456.5	275	593.1
E+2	128	180.6	197.5	197.4	96.5	177	120	208.4
SUM	536	724.8	957.5	749.7	634	829.3	583	1112.3
MEAN	178.7	241.6	319.2	249.9	211.33	276.43	194.33	370.76
STD	71.14	103.58	171.72	111.08	105.85	156.23	77.69	199.24
P<0.005 is significant		0.086	0.137	0.113	0.439	0.186	0.375	0.123
			0.196	0.420	0.534	0.373	0.223	0.159

stretchy, cloudy. The score; Consistency, 10 scored (4), color, cloudy is scored (2), Changed to nonlubricative scored (2), sensation nonlubricative scored (0). Total = 4 + 2 + 2 + 0 = 8. The sperm survival in the cervical mucus is very important in natural reproductive medicine and so limited or dry mucus scores are associated with some form of fertility challenge.

The schematic representation in Figure 1 is what is used in NPT to target when to collect blood samples for hormonal assays to determine the periovulatory Estrogen levels, and postovulatory estrogen and progesterone. The way to do accurate targeting is to first look at the previous complete chart the woman has made, for example the chart in Figure 6a, above. Identify the peak day and count backward from the peak-1 to peak-6. In Figure 6a, the peak day is on the 19th day of the cycle, therefore 6-steps backwards will be day 13th. Then in the new cycle which the woman will make prospectively, the 13th day will be peak-6, 15th day will be peak-4, 17th day will be peak-2, 19th day will be peak day, and 21st day will be peak +2. And, in all these days, blood will be collected for periovulatory estrogen assessment. Also, it is on those days that targeted ultrasound will be done for follicular tracking. During ultrasound pay attention to measuring the length, breath, and transverse diameters of the developing follicle and calculate the MFD = (L + B + T)/3 in mm or cm. Also, pay attention to identify the presence or absence of cumulus oophorus. By the time you get to the predicted peak +2 in the cycle of evaluation, the woman would have identified the peak day in that very cycle. And with the peak day identified, we can then identify the days for peak +3, +5, +7, +9, and +11 for the collection of postovulatory blood samples for estrogen and progesterone. The effectiveness of this method to target the hormones accurately is 96.9%.[12] The normal range used in this study was from a study done on 57 women at the Pope Paul VI Institute for the Study of Human Reproduction in Omaha USA. They were all regularly ovulating with positive cumulus complex present in matured follicles (18–26 mm).[13] In all ultrasound diagnosed ovulation, the follicular rupture is complete with follicular rupture difference (FRD) ≥7.5 mm. The assumption in the study at Omaha was that since these 57 women had satisfactory ultrasound diagnosed ovulation of matured follicles with positive cumulus complex, they also

therefore must correspond with normal periovulatory estrogen, and postovulatory (postpeak) estrogen and progesterone values.

Based on this graph in Figure 5, the simplified working data for maximum matured follicle size in "mm" for age at which HCG trigger could be given to induce follicular rupture included the following:

23 Yr(24.9), 24 Yr(24.7), 25 Yr(24.5), 26 Yr(24.2), 27 Yr(24.0), 28 Yr(23.8), 29 Yr(23.6), 30 Yr(23.5), 31 Yr(23.3), 32 Yr(23.1), 33 Yr(23.0), 34 Yr(22.8), 35 Yr(22.5), 36Yr(22.4), 37 Yr(22.2), 38 Yr(22.0), 39 Yr(21.7), 40 Yr(21.5), 41 Yr(21.3), 42 Yr(21.0), 43 Yr(20.8), 44 Yr(20.5), 45 Yr(20.3), 46 Yr(20.0), 47 Yr(19.8), 48 Yr(19.6), 49 Yr(19.4), 50 Yr(19.0).

Conversion to IS units:

- Estrogen: ng/dl × 10 = pg/ml; pg/ml × 3.671 = pmol/ml
- Progesterone: nmol/l × 0.314 = ng/ml; ng/ml × 3.18 × 1000 = pmol/ml.

Table 5b: Study of the luteal phase estrogen (pg/ml) in the omaha, control Group A and Group B

	OMAHA	Control GROUP A	GROUP B
P+3	88	224.6	219.2
P+5	108	291.5	241.6
P+7	120	243.9	278.7
P+9	120	299.1	247.9
P+11	98	227.3	195.1
SUM	534	1286.4	1182.5
MEAN	106.8	257.3	236.5
STD	13.97	35.59	31.42
P<0.005 is significant		0.000296	0.000189
			0.268

Table 5c: Study of the luteal phase progesterone (ng/ml) comparing omaha, control Group A and Group B

	OMAHA	Control Group A	Group B
P+3	9.4	20	9.65
P+5	14.4	24.7	11.91
P+7	15.7	25.1	10.62
P+9	13.6	22.6	8.86
P+11	8.1	12.3	6.86
SUM	61.2	104.7	47.9
MEAN	12.24	20.94	9.58
STD	6.17	5.24	1.89
P<0.005 is significant		0.0017	0.059
			0.0022

Presentations of Results

The results of the study are presented in Tables: 1a, 1b, 2a, 2b, 3, 3a, 3b, 4a, 4b, 5a, 5b, 5c, 5d and Figure 5.

Discussion

From Table 1a, the Control Group (A), a total of 15 cycles were studied. And, a total of 15 follicles were also studied. Moreover, based on NPT classification of ultrasound ovulation diagnosis, only one follicle did not reach the minimum matured size of 19.0 mm,[14] it was 18.8 mm, but was considered matured by Ojengbede *et al*.[2] All the cycles were regular cycles ranging from 25 to 30 days. The age ranges was from 23 to 45 years with a mean age of 30.5 years; standard deviation (SD) 7.76. The maximum matured follicles ranges from 18.8 to 24.9 mm, with a mean size of 20.6 mm, SD 1.62. Standard graph was drawn using data from the control group to develop the Zone ABove the line (AB), the Zones On the maximum Line (OL), and the Zones BeLow the line (BL). The size of the maximum matured follicles was on the Y-axis, while the age of the women was on the X-axis [Figure 5]. In the Control Group (A), 13 cycles recorded the matured MFS below the line (BL) (86.7%); and 2 cycles recorded the matured MFSs on the control line (OL) (13.3%). We also noticed that the maximum matured follicular size synchronized with the peak periovulatory estrogen (PE2) 100% of the time. Our study from the fertile control Group (A) seems to suggest that large matured follicles are good for younger women, and smaller size matured follicles are good for older women.

In NPT, ovulation defects could be diagnosed based on FRD [Figure 7]. A complete follicular rupture is when the

Table 5d: Study of luteal phase E2/Pg ratio comparing omaha study with control Group A, Group, Group A (OL), Group A (BL), Group B (AB), Group B (OL), Group B (BL)

	Omaha	Group A	Group A (OL)	Group A (BL)	Group B	Group B (AB)	Group B (OL)	Group B (BL)
P+3	9.36	11.23	6.39	12.49	28.73	30.65	41.67	26.44
P+5	7.5	11.8	5	14.03	20	22.54	27.78	10.12
P+7	7.64	9.72	5.43	10.54	25.96	27.78	43.75	22.28
P+9	8.82	13.2	17.01	12.59	28.15	32.42	33.33	25.44
P+11	12.09	18.48	25.83	18.59	28.35	36.01	46.05	23.93
SUM	45.41	64.43	59.66	68.24	131.19	149.4	192.58	108.21
MEAN	9.082	12.886	11.932	13.648	26.238	29.88	38.516	21.642
STD	1.855807	3.366627	9.219312	3.028584	3.6509	5.072154	7.685374	6.630311
P<0.005 is significant		0.010415	0.452666	0.004839	0.000187	0.000204	0.000614	0.009052
			0.746784	0.191599	0.001865	0.000448	0.001521	0.046157

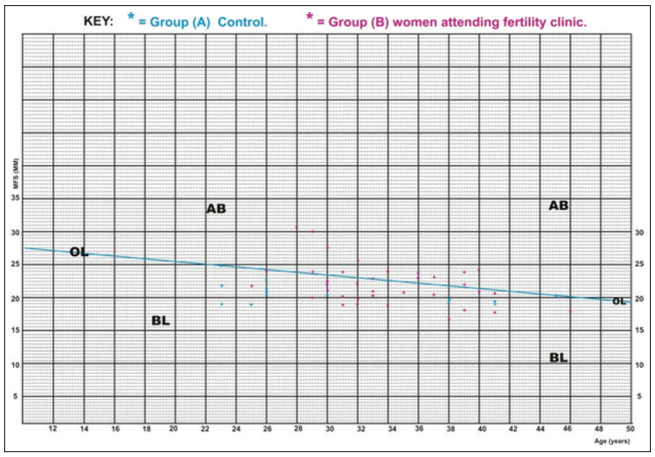

Figure 5: Graph of the MFS (y-axis) against the age of the women (x-axis) and connecting the highest follicular size attained by the youngest woman in control (A) to the highest follicular size attained by the oldest woman in control (A). Follicles above the drawn line are in Zone AB, follicles below the line are in Zone BL. Follicles that fall on the line are in Zone OL

FRD is ≥7.5 mm. Calculating FRD is done by this formula: (MFS − RFS = FRD). From Table 1a, ten cycles (66.7%) had complete follicular rupture, while five cycles (33.3%) had partial rupture syndrome (PRS). Details of the periovulatory estrogen and postovulatory progesterone at peak +7 are summarized in Tables 1b, 3a-b, and 5a.

Out of the 13 cycles that are BL, 9 (69.2%) had normal periovulatory estrogen, and out of these 9, 6 (66.7%) had high mid luteal (*P* + 7) progesterone with associated 100% complete follicular rupture, while the remaining 3 (33.3%) had normal mid luteal (*P* + 7) progesterone with associated 66.7% PRS, and 33.3% complete rupture.

Four of the BL cycles (30.8%) had high periovulatory estrogen, and out of these four, one (25%) had high mid luteal (*P* + 7) progesterone with associated 100% complete follicular rupture, while the remaining three (75%) had normal mid luteal (*P* + 7) progesterone with associated 100% PRS.

Out of the two cycles that are OL, one had high periovulatory estrogen, while the other had normal periovulatory estrogen. They both had high mid luteal (*P* + 7) progesterone and both associated with complete follicular rupture.

From the control Group (A), complete follicular rupture seems to be associated with high mid luteal progesterone outcomes while PRS seems to be associated with normal mid luteal (*P* + 7) progesterone irrespective of whether the periovulatory estrogen was high or normal [Table 1b].

Among the women attending Fertility Clinic, Group (B), a total of 37 cycles were studied. All the cycles were regular cycles ranging from 24 to 31 days. The ages ranged from 25 to 46 years, with a mean age of 33.7 years, SD 4.84. The matured MFSs ranges from 18.0 to 31.1 mm, with a mean size of 22.4 mm, SD 3.19. The mean MFS in the women attending fertility clinic Group (B) of 22.4 mm is significantly bigger ($P = 0.0043$) compared to the control Group (A) with mean MFS of 20.6 mm. We also noted that the average age of the control Group (A) of 30.5 year was not statistically different from the average age of the Group (B) of 33.7 years ($P = 0.075$).

In Group (B) 37 cycles was studied in 37 women attending fertility clinic. One woman had two follicles that matured and completely ruptured and therefore a total of 38 follicles were studied. Using the graph in Figure 5 we found out that the 38 follicles studied in these 37 women fell into 3 categories; Group B (AB) 14 cycles (36.8%), Group B (BL) 23 cycles (60.5%), and Group B (OL) one cycle (2.6%). Of the 38 follicles studied, 5 did not attain NPT minimum matured size of 19.0 mm (they were 16.9, 17.7, 18.0, 18.9, 18.9),[14] but they are considered matured based on the Ojengbede *et al*. studies.[2] Complete follicular rupture occurred in 30 follicles (78.9%), and PRS occurred in 8 follicles (21.1%). Details of the periovulatory estrogen and postovulatory progesterone at peak +7 were studied in Tables 2a, 4a- b and 5a.

Out of the 14 cycles that are AB cycles in Group (B), 12 (85.7%) had high periovulatory estrogen, and out of these, 8 (66.7%) had low mid luteal (*P* + 7) progesterone with associated 87.5% complete follicular rupture, and 12.5% PRS; while the remaining 4 (33.3%) had normal mid luteal (*P* + 7) progesterone with associated 75% complete follicular rupture, and 25% PRS.

One cycle of AB (7.1%) out of the 14 cycles had normal periovulatory estrogen with a normal mid luteal (*P* + 7) progesterone and associated complete follicular rupture. And one cycle of AB (7.1%) out of the 14 cycle had low periovulatory estrogen with a low mid luteal (*P* + 7) progesterone with associated complete follicular rupture.

Despite the high percentage of complete rupturing of follicles in the AB cycles, there is very high proportion having low mid luteal (*P* + 7) progesterone. This may be due to pathologies associated with big follicles for woman's age. And also, high periovulatory estrogen in these big follicles seems to contribute to their large size. There is a possibility that cumulus complex defect may be associated as shown in ovulation defect studies by Hilgers.[15]

Out of 23 cycles that are BL cycle in Group (B), 11 (47.8%) had high periovulatory estrogen, and out of these, 6 (54.5%) had normal mid luteal (*P* + 7) progesterone with associated 83.3% complete follicular rupture, and 16.7% PRS; while the remaining 5 (45.5%) had low mid luteal (*P* + 7) progesterone with associated 80% PRS and 20% complete rupture.

Out of the 23 cycles that are BL in Group (B), 9 (39.1%) had normal periovulatory estrogen, and out of these, 5 (55.6%) had normal mid luteal (*P* + 7) progesterone with associated 100% complete follicular rupture; and another 3 (33.3%) had low mid luteal (*P* + 7) progesterone with associated 100% complete follicular rupture; and the last one cycle (11.1%) had high mid luteal (*P* + 7) progesterone with associated complete follicular rupture. (We expected the three follicles [33.3%] with

normal periovulatory estrogen and 100% complete rupture to have at least normal mid luteal [*P* + 7] progesterone but they had low values. These follicles may be empty follicles without cumulus. Further research is needed to get better insight of what happened).

Out of the 23 cycles that are BL in Group (B), 3 (13.0%) had low periovulatory estrogen, and out of these, 2 (66.7%) had low mid luteal (*P* + 7) progesterone with associated 50% complete rupture, and 50% PRS; and one cycle (33.3%) had low periovulatory estrogen, and normal mid luteal (*P* + 7) progesterone with associated complete follicular rupture.

In the BL cycles in Group (B) with either high or normal periovulatory estrogen, complete rupture leads to normal mid luteal (*P* + 7) progesterone while PRS lead to low mid luteal (*P* + 7) progesterone.

In the Group (B) women, only one cycle (2.6%) was OL. And, this cycle had normal periovulatory estrogen, with low mid luteal (*P* + 7) progesterone with associated complete rupture. We expected this follicle to have at least normal mid luteal progesterone but it is possible that the size has already started to have adverse effect on the follicular function and cumulus complex defect may also be involved. Further research is needed to get deeper insight.

We must note that in NPT, ovulation defects is also considered with regards with the presence or absence of cumulus complex in a follicle, and also even examined if the cumulus complex was released or retained after rupture event.[15] We did not look into cumulus complex study because of limitation to do the very technical ultrasound involved. So it is a possibility that some of the matured follicles in the Group (B) may also have cumulus challenges resulting in poor luteal progesterone. Further research in this regard is needed.

In Table 3, studying the Group (B), the distribution of AB, BL, OL, by age ranges showed that the cycles recording AB dominated 60% only among age range 34–36 years. From the study, we noted that AB cycle can occur from ages 28 to 42 years, and it is important to be vigilant about this in managing client during fertility assessment.

The hormonal assay results both for periovulatory estrogen and postovulatory estrogen and progesterone in Group A and subgroups namely Group A (BL) and Group A (OL) and in Group B and subgroups namely Group B (BL), Group B (OL), and Group B (AB) are all presented in Tables 3a-b, 4a-b, and 5a. The statistical study of these hormones and how they were compared with the Omaha study are reported in Table 5a-d.

In Table 5a, the control Group A and Group B, with all their subgroups had high periovulatory estrogen sum of 3-values compared to the Omaha study, but none reached statistical significance.

In Table 5b, both the Group A ($P = 0.000296$) and Group B ($P = 0.000189$) had significantly increased luteal phase estrogen sum of 5-values compared to Omaha study.

In Table 5c, Group A ($P = 0.0017$) had significant increase in luteal phase progesterone sum of 5-values; while the Group B ($P = 0.059$) had reduced luteal phase progesterone sum of 5-values but was not statistically significant compared to the Omaha study. However, the Group B reduced luteal phase progesterone sum of 5-values was compared with the Group A control, the reduction was very significant ($P = 0.0022$).

In Table 5d, the estrogen/progesterone ratio (E2/Pg) was studied for Group A, and subgroups namely; Group A (BL), Group A (OL), and Group B and subgroups namely; Group B (BL), Group B (OL), Group B (AB). Note that the estrogen unit was (pg/ml), and progesterone unit was (ng/ml). The study of E2/Pg ratio is a better assessment of the luteal phase status rather than just checking the individual hormones. A high E2/Pg ratio indicates estrogen dominance in the luteal phase, while a low E2/Pg ratio indicates progesterone dominance in luteal phase. Estrogen dominance in luteal phase is associated with narrow or closed implantation window. According to a study published in the Proceedings of the National Academy of Science of the United States of America, "the window of uterine receptivity remains open for an extended period at lower estrogen levels but rapidly closes at higher estrogen levels."[16] Looking at the statistic in Table 5d, we noticed that Group B (AB) had the most significant estrogen dominance in luteal phase. The Group B (BL) did not show statistical significance in increase in the E2/Pg ratio compared to Omaha study or to Group A control. This shows that the women in this group had relatively favorable implantation window compared to those in Group B (AB). It is important to note that the 26-year-old woman in Group B (BL) cycle 15, had a MFS of 24 mm and she conceived in the cycle of her evaluation!

Conclusion

In both the control group (Group A) and the women attending Fertility Clinic (Group B), we discovered that the MFS reduces as the woman's age increases. We also discovered that the average MFS in the Group (B) (22.4 mm) was higher, with $P = 0.0043$, compared with the control Group (A) (20.6 mm). Our study from the fertile control Group (A) and the Group (B) women seem to suggest that large matured follicles are healthy for younger women, and smaller size matured follicles are healthy for older women. In an *in vitro* fertilization (IVF) study by Jaime *et al*., they discovered that large leading follicles do not yield a higher percentage of matured oocytes, and they even resulted in a lower, albeit not significant so, live birth rate.[17]

From the control Group (A), complete follicular rupture was associated with high mid luteal progesterone outcomes while PRS was associated with normal mid luteal (*P* + 7) progesterone irrespective of whether the periovulatory estrogen was high or normal. These suggest that the cumulus complex activities in these normal controls are functioning well, but further study is needed to understand the phenomenon better.

Despite the high percentage of complete rupturing of follicles in the AB cycles in the Group (B) there are very high proportion

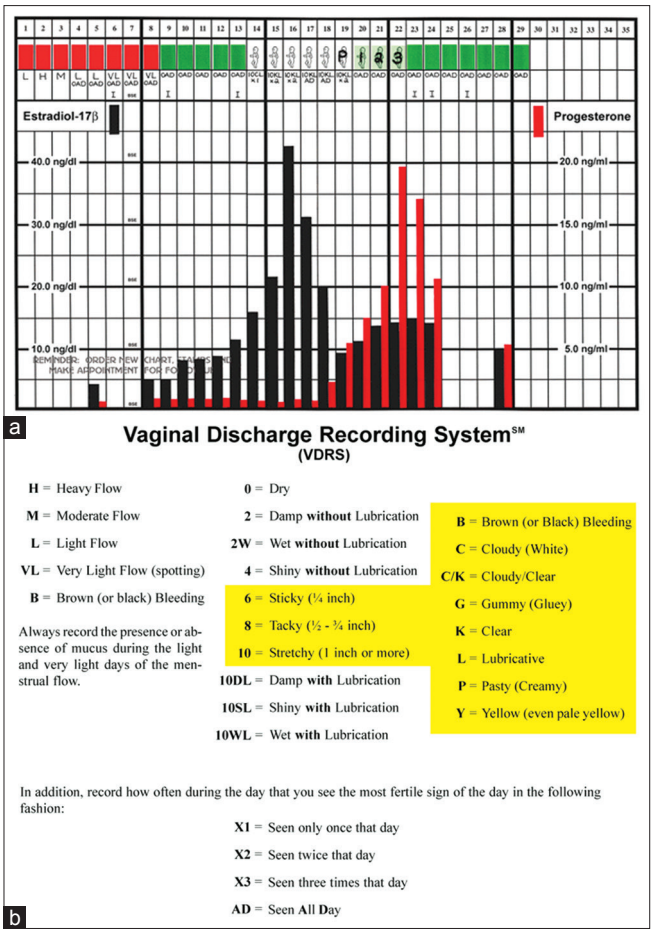

Figure 6: (a) The relationship of serum levels of estradiol-17 beta and progesterone during the course of the menstrual cycle and the occurrence of the mucus sign and the peak day (*P*) in one cycle of a woman with normal fertility. (b) The Vaginal Discharge Recording System is at the back of the Creighton Model FertilityCare System chart that clients are given at the fertility centers after attending "IS." These coded representations of the various vaginal discharges are well understood by women after they have been instructed by a trained fertility care practitioner

having low mid luteal (*P* + 7) progesterone. This may be due to pathologies associated with big follicles for woman's age. And also, high periovulatory estrogen in these big follicles seems to contribute to their large size. Possibility of cumulus complex defect association should be considered.[15]

In the BL cycles, among the Group (B) with either high or normal periovulatory estrogen, complete rupture leads to normal mid luteal (*P* + 7) progesterone while PRS lead to low mid luteal (*P* + 7) progesterone. This was not what was observed in the BL cycles in the control Group (A). Therefore, the PRS cases in the Group (B) may also have associated cumulus complex defects leading to the low mid luteal (*P* + 7) progesterone.

The E2/Pg ratio study in luteal phase strongly suggested that the women in Group B that may have fertility challenge are those in subgroup of Group B (AB). Therefore, matured follicular size should be considered with the woman's age. If the follicles

Summary of Reproductive Anomalies Clinically associated with Disorders of Ovulation

Ovulation Disorder	Reproductive Anomalies
Luteinized unruptured follicle (LUF: +, –). No rupture at all.	Absolute infertility
Afollicularism (AF), <14mm size	Absolute infertility
Immature follicles,>15, <19mm (IFS: +, –, Re)	Relative infertility and abnormal pregnancies
Partial rupture, FRD<7.5mm (PRS: +, –, Re)	Relative infertility and abnormal pregnancies
Empty follicle syndrome, MF no CO	Relative infertility
Delayed rupture complete over 48hr (DRS: +, –, Re)	Needs further study

Figure 7: This shows the various ovulation defects that could be diagnosed by ultrasound in NaProTECHNOLOGY

grow bigger away from the range for a woman's age, it may be the marker for infertility or reproductive health challenges.

Recommendation

We hypothesize that this large follicle for age may be linked with infertility and possible miscarriages. The IVF studies of Jaime *et al*. even though did not consider woman's age did suggest that large follicles are linked with low birth rate.[17] We propose further studies to verify this hypothesis. We recommend that trigger HCG be given to women at the point that will enable them rupture their maximum matured follicles within the sizes that falls below, or exactly on the control line for their age in order to improve their fertility and chances of conception. This suggestion is a simplistic derivation from the graph in Figure 5. We recommend that, in case of bigger matured follicles for age with associated high periovulatory estrogen, aromatase inhibitors and anti-low-density lipoprotein cholesterol be given during the periovulatory period to bring the estrogens to the normal range and also control the development of bigger matured follicles for age. Finally, we recommend that cumulus complex study be included during routine ultrasound follicular tracking so as to have a comprehensive anatomical ovulation diagnosis.

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Conflicts of interest

There are no conflicts of interest.

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