Original Article

Evaluation of Effects of Artemether + Lumefantrine (Artemisinin-based Combination Therapy) on Women's Reproductive Cycle using Creighton Model FertilityCare System and NaProTECHNOLOGY

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Abstract

Background: Malaria is prevalent in Nigeria, and artemether + lumefantrine (artemisinin-based combination therapy [ACT]) is drug of choice in treatment of uncomplicated cases. ACT is contraindicated in early pregnancy. They release-free radicals that can compromise female fertility. Infertility and its associated complications such as miscarriages, abnormal gestation, and unstable marriages seem to be on the increase. This study aims at evaluating the effect of ACT on female fertility. The significance of this research is to draw the attention of fertility care givers to this possible cause of infertility and fertility challenges. **Subjects and Methods:** Creighton Model FertilityCare System and NaProTECHNOLOGY are simply technologies that can be used to assess female fertility. They are used in this study to assess the effect of ACT administered at different stages of menstrual cycles of three selected fertile adult females. The results are interpreted on the background of standard Creighton model chart. **Results:** This study has shown that ACT has a significant fertility deteriorating effect on the women. It caused ovulation defect and diagnosed as partial rupture syndrome in the very cycle of use and in the first cycle after use. It also significantly reduced cervical mucus production and significantly reduced luteal phase progesterone production with an associated significant increase of luteal phase estrogen production. **Conclusion:** ACT use as antimalarial may be a possible cause of infertility and fertility challenges in women.

Keywords: Artemether + lumefantrine, Creighton Model FertilityCare System, infertility, NaProTECHNOLOGY

Introduction

Artemether + lumefantrine (artemisinin-based combination therapy [ACT]) is prescribed drug of choice in treatment of uncomplicated malaria in Nigeria.[1] Malaria is prevalent, "there are an estimated 100 million malaria cases with over 300,000 deaths/year in Nigeria."[2] "Lumefantrine binds to heme produced during hemoglobin breakdown, preventing detoxification to crystalline malaria pigment (hemozoin). During the same process, the peroxide group in artemether binds to heme and releases toxic-free radicals."[3] Dennery and Sharma and Agarwal have reported adverse effects of reactive oxygen species (OS) on reproductive system.[4,5] OS in female reproduction may be a major link in the infertility puzzle as well as in some reproductive organ diseases such as endometriosis. Recently, OS has been reported to have an important role in the normal functioning of the female reproductive system and in the pathogenesis of female infertility.[6,7]

"Long-term administration of artesunate induces reproductive toxicity in male rats; the results suggest that long-term administration of artesunate could induce reversible infertility in rats which may act through distortion of blood–testis barrier formed by Sertoli cells."[8] Abolaji *et al*. attempted to ascertain the contraceptive claim of *Artemisia Annua,* and their results showed that *A. annua* (*A. annua* L is a common type of wormwood that belongs to the family of the *Asteraceae*). It is native to temperate Asia but naturalized throughout the world. Artemisinin is an ingredient of

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A. annua. Artemisinin and its semi-synthetic artemisinin derivatives (including dihydroartemisinin, artesunate, artemether, and arteether) are used for the production of combination therapies for treatment of malaria (ACTs) significantly reduced litter size, reproductive outcome, and fertility indices compared with the control ($P < 0.05$), implying that *A. annua* could serve as a prospective contraceptive agent in addition to its antimalarial activity.[9] Obianime and Aprioku did a comparative study of effects of artesunate, ACTs on Guinea-pig. The results of this study showed that the agents caused significant decreases ($P < 0.05$) in serum testosterone level, with varying effects on luteinizing hormone (LH), follicle-stimulating hormone, and prolactin.[10] Most antimalarial agents have been reported to possess various degrees of antifertility activities.[11,12]

The generation of free radicals appears to be the major way they cause fertility challenge. The uniqueness of the Creighton Model FertilityCare System (CrMS) and NaProTECHNOLOGY (NPT) is that it telegraphs the woman's reproductive cycle on a daily basis in a prospective way, enabling easy evaluation of the various phases of the reproductive cycle.

The effects of ACT on fertility in experimental animals have been studied in literature, but little is known of its effect on human female fertility. The aim of this research is to evaluate the effects of ACT on fertility of selected fertile females attending St. Margaret's Hospital and Maternity Felele Phase 1, Lokoja, using CrMS and NPT.

Saint Margaret's Hospital and Maternity is a fully registered private hospital in Kogi State. The hospital started as a Clinic in Ogori-Magongo/Okene in 1999–2015 before eventually established as Saint Margaret's Hospital and Maternity in 2015 and relocated to permanent site in Lokoja, the capital of Kogi State. Within the hospital, we offer the Saint Margaret's Natural Procreative Technology (NPT) and CrMS services. Our vision is to expand the Saint Margaret's NaProFertility Care Center, based in Kogi State Nigeria with the intention to establish it as the African center focusing on natural reproductive care and as well as research and training.

Subjects and Methods

Subjects

Three women were involved in the study. These three women were selected by convenience after confirming that they were regularly ovulating with ultrasound diagnosis of matured follicle (18–26 mm) that always ruptures with a rupture difference >7.5 mm. No previous history of ACT used in the past 6 months before the study. The purpose of the study was explained to them, and they all gave verbal consent. All three women had regular menstrual cycle ranged from 27 to 30 days. There was no financial inducement to them. They all participated to support the reproductive research in the center. Each woman was permitted to stop coming for ultrasound, hormonal assay, or CrMS follow-up if they feel they cannot continue.

Study duration was May–December 2014.

- Woman "A" is 23 years old, single, and two cycles was studied (cycle without ACT and cycle with ACT). November and December 2014
- Woman "B" is 23 years old, single, and three cycles studied (cycle without ACT, cycle with ACT, and first cycle post-ACT). May, October, and November 2014
- Woman "C" is 38 years old, married, and four cycles studied (cycle without ACT, cycle with ACT, first cycle post-ACT, and second cycle post-ACT). August, October, November, and December 2014.

The methods used to assess the effect of ACT on female fertility included:

- Analysis of the charting pattern of the CrMS before and after the use of ACT (paying attention to the biomarkers)
- Targeted hormonal assay before and after the use of ACT (artemether + lumefantrine)
- Targeted serial ultrasound scanning trans abdominal (TA) before and after the use of ACT (artemether + lumefantrine)
- ACT was given to them in the cycle of positive malaria parasite test confirmed by thick film. Medication administered from 10th to 12th day of cycle (based on the day growing or matured follicle was noted by ultrasound in particular woman) and lasted 3 days. We wanted to see the effects of ACT on follicle, so we ensured there was follicle growing before using the ACT
- Dosage: Tablet 560 mg (artemether + lumefantrine ACT) stat, then 560 mg after 8 h; then 560 mg BD × 2 days from next day. No other medication added.

Creighton Model FertilityCare System and NaProTECHNOLOGY

In Figure 1a, we see 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 fertility care practitioner (FCP). This IS lasts between 4 and 6 h 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 good FCP will give the necessary motivation, while the spouses 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 daily basis. The "RED" stamp is to mark days

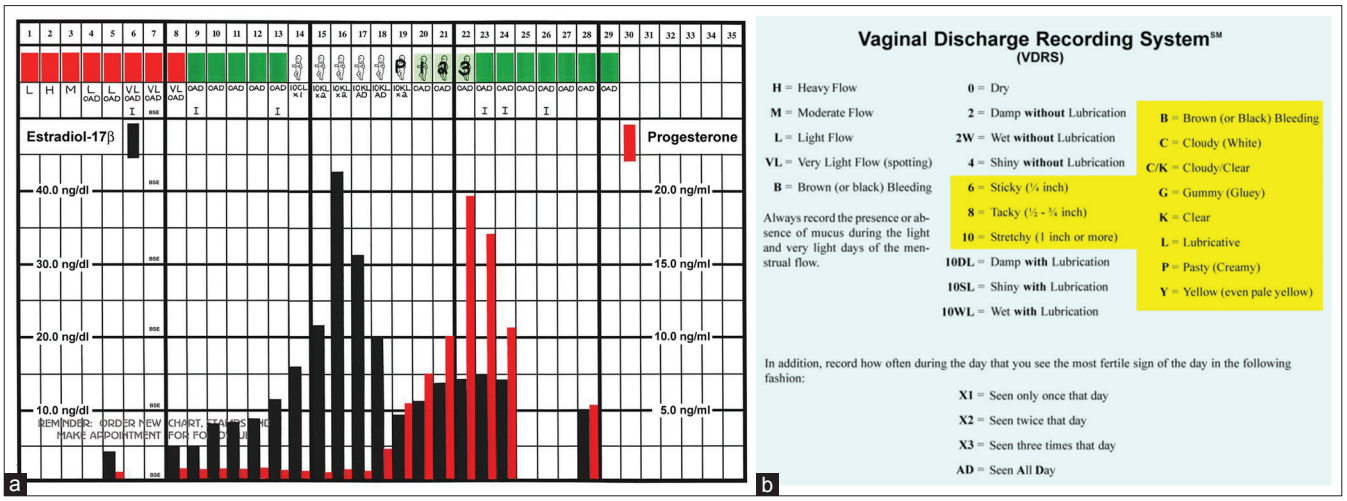

Figure 1: (a) The relationship of serum levels of estradiol-17b and progesterone during the course of the menstrual cycle and the occurrence of the mucus sign and the Peak day (P) in one cycle of a woman with normal fertility. (b) The vaginal discharge recording system is at the back of the Creighton Model FertilityCare System chart that clients are given at the fertility centers after attending "introductory session." These coded representations of the various vaginal discharges are well understood by women after they have been instructed by a trained fertility care practitioner

of bleeding, which will include menstrual flow or any other bleeding event that may occur. Any day she sees blood must have RED stamp irrespective what day in her cycle it occurred. The numbers 1–35 on top of the chart represents days of cycle and not 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 29th of July, then day 1 on the chart will be dated 29/07, and day 2 will be 30/07, and so on. Moreover, immediately below the date space is the space for recording of the observation using the code of the vaginal discharge recording system [Figure 1b].

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; example in Figure 1a, she has dryness from 9th to 13th day of her cycle in the pre-Peak phase (follicular phase) and dryness from 20 to 29th day of her cycle in the post-Peak phase (luteal Phase) of her cycle. Technically, pre-Peak phase (day 1–19) is from the 1st day of menstrual flow up until including the peak day. While the post-Peak phase (day 20–29) is from the 1st day after the Peak day till the last day before the beginning of the next menstrual period. CrMS study has revealed that the post-Peak phase is relatively constant in length (12 ± 4 days), whereas the pre-Peak 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 is to use the "White Baby" stamps. Moreover, the mucus could be a Peak type or non-Peak 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 non-Peak type mucus is the mucus discharges that are not clear, not stretchy, and not lubricative.

The "Green Baby" stamps are special stamps used to indicate the period after the Peak day when the cervix is closing with dryness but still fertile. 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 Peak ±3.[13] The cervical mucus, which the woman observes, is a reflection of the response of the cervix to the estrogen which the developing follicles produce. Moreover, NPT has developed a standardized scoring system for the mucus which women observe.[14,15] 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), and + (Peak day) is calculated and the sum divided by 6. The mucus is scored on four characteristics (3CS), namely, The cervical mucus is scored on four characteristics (3CS), namely : the Consistency, the Color ,the Sensation ,and finally the Change that occurs within the mucus . (1) consistency (length of mucus), sticky mucus is coded 6, and Tacky mucus is coded 8,and both have mucus score of 2. The Stretchy mucus is coded as 10, and it has mucus score of 4. (2) Color, mucus that is brown, cloudy, or yellowish in color is scored 2.The mucus that is crystal clear, transparent in color 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), color, cloudy is scored (2), changed to lubricative scored (4), sensation is lubricative scored 4. Total = 2 + 2 + 4 + 4 = 12. Example 2: 10C means stretchy, cloudy. The score; consistency, 10 scored (4), color, cloudy is scored (2), changed to nonlubricative X 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 drawing in Figure 2 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 1a. Identify the Peak day and count backward from the Peak 1 to Peak 6. In Figure 1a, the Peak day is on the 19th day of the cycle; therefore, six steps backward 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. Moreover, in all these days, blood will be collected for periovulatory estrogen assessment. Furthermore, 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 mean follicular diameter = (L + B + T)/3 in mm or cm. Furthermore, 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. Moreover, 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%.[16] 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).[17] 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 (post-Peak) estrogen and progesterone values see details of these normal values in Figures 3-6.

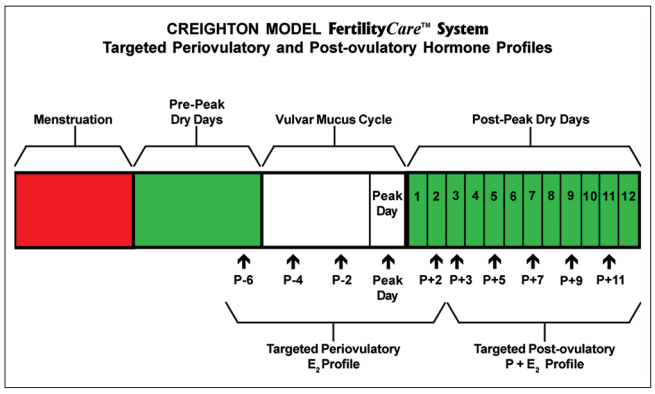

Figure 2: This schematic drawing of the Creighton Model FertilityCare System demonstrates how it is used to target the periovulatory and postovulatory hormone profiles

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.

Estrogen/progesterone ratio

In luteal phase, both estrogen and progesterone are produced. The ratio of the E2/Pg is calculated and used to study the luteal phase better. Isolated assessment of luteal phase estrogen alone without connecting with the corresponding luteal phase progesterone may give some misleading interpretations. When E2/Pg ratio is increasing, it is an indication of estrogen dominance in luteal phase and that compromises the implantation window, and also when the E2/Pg ratio is reducing in luteal phase, it is an indication of improving implantation window and improving fertility.[18]

Discussion

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).[17] In all ultrasound diagnosed ovulation, the follicular rupture is complete with FRD ≥7.5 mm. The

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 3: 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 Peak − 5 (or Peak − 6) through Peak + 2 (with reference to the Creighton Model FertilityCare™ 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 Table 24-2

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 4: The various ovulation defects that could be diagnosed by ultrasound in NaProTECHNOLOGY. The ovulation defects associated with artemisinin-based combination therapy is partial rupture syndrome. The connotation (+) presence of cumulus oophorus; (−) absent of cumulus oophorus; (Re) retained cumulus oophorus. Abnormal pregnancies includes (ectopic gestation, miscarriage, congenital anomalies, prematurity, etc.,)

Table 24-5: Mean and Standard Deviation of Post-Peak Estradiol-17β Profile[1,2] and E_2 Sum and Mean Normal Controls[3] (N=57)

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 6: The post-Peak estrogen from the study of 57 normal ovulating women with evidence of cumulus oophorus and follicular rupture ≥7.5 mm

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 (post-Peak) estrogen and progesterone values. These normal values are included in brackets (), with data in Figures 7 and 8.

In Figures 9-18, the CrMS charts of woman "A", woman "B", and woman "C" were represented showing respectively their various cycles of No ACT, cycles of ACT Used, and

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 5: 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) Table 24-3

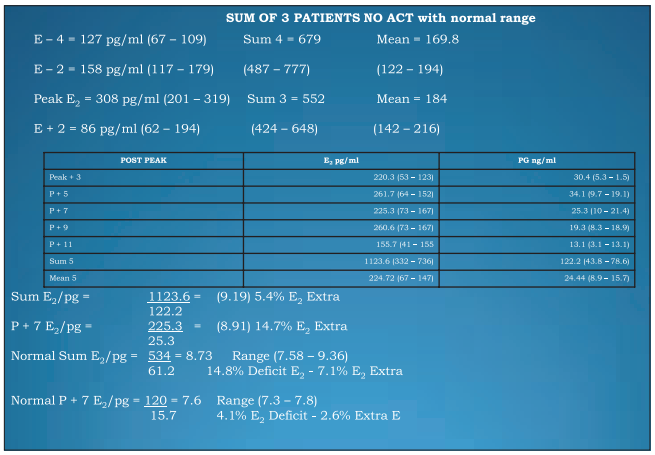

Figure 7: The sum of the three women showing the follicular phase estrogen and luteal phase progesterone and estrogen and comparing with normal range in the cycle of no artemisinin-based combination therapy

cycles After ACT Used. These Figures 9-18 also shows hormonal assay of FSH, Estrogen and Progesterone, with serial ultrasound scan and follicular rupture difference (FRD), and Endometrial thickness. In Figure 19, we compared the estrogen produced in follicular phase between normal, no ACT, and ACT cycles. The sum of the three patients was used. No ACT versus normal (P = 0.47442). ACT versus normal (P = 0.177102). ACT versus no ACT (P = 0.22498). Even though there was no statistical significance, there was relatively more estrogen produced in the follicular phase of the ACT cycle. When we looked at Figure 20, the cervical mucus produced in the No ACT cycle compared to the ACT cycle, it revealed that there was a significant reduction of the cervical mucus in the ACT cycle (P = 0.019). In NPT, a very reliable system has been developed that helped in the objective scoring of the cervical mucus which women observe at the vulva.[19] Since the ACT cycle had more estrogen produced in

SUM OF 3 PATIENTS ACT CYCLE

E – 4 = No Data
E – 2 = 192.7 (117 – 179)
Peak E_2 = 397.7 (201 – 319)
E + 2 = 199.7 (62 – 194)

Sum E_2 of 3 = 790.1 (424 – 648)
Mean $E_2$3 = 263.4 (142 – 216)

POST PEAK	E_2 pg/ml	PG ng/ml
Peak + 3	471.7 (53 – 123)	13.7 (5.3 – 13.5)
P + 5	580 (64 – 152)	21 (9.7 – 19.1)
P + 7	225 (73 – 167)	11 (10 – 21.4)
P + 9	426.7 (73 – 167)	7 (8.3 – 18.9)
P + 11	386.7 (41 – 155)	6.3 (3.1 – 13.1)
Sum 5	2, 090.1 (332 – 736)	59 (43.8 – 78.6)
Mean 5	418.02 (67 – 147)	11.8 (8.9 – 15.7)

Sum $\frac{E_2}{Pg} = \frac{2090.1}{59}$ = (35.4) = 75.4% E_2 Extra

P + 7 $\frac{E_2}{Pg} = \frac{225}{11}$ = (20.5) = 62.8% E_2 Extra

Figure 8: The sum of the three women showing the follicular phase estrogen and luteal phase progesterone and estrogen and comparing with normal range in the cycle of artemisinin-based combination therapy use

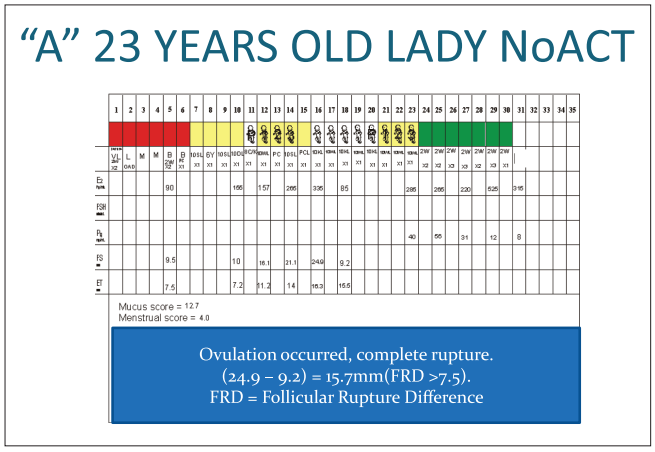

Figure 9: This shows that woman "A" completely ruptured matured follicle around the 18th day of her cycle. Regular mucus score 12.7, and periovulatory estrogen and postovulatory estrogen and progesterone are within normal range. In this cycle, artemisinin-based combination therapy was not used

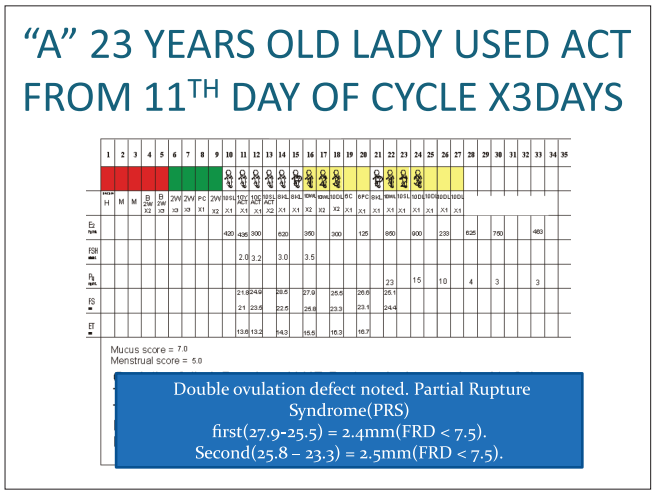

Figure 10: This shows what happened to woman "A" after the use of artemisinin-based combination therapy from the 11th day of her cycle. She had double matured follicles that both experienced ovulation defect (partial rupture syndrome). The mucus score reduced to limited mucus score of 7.0. The follicular and luteal phase estrogen increased, with a corresponding highly significant reduction of luteal phase progesterone

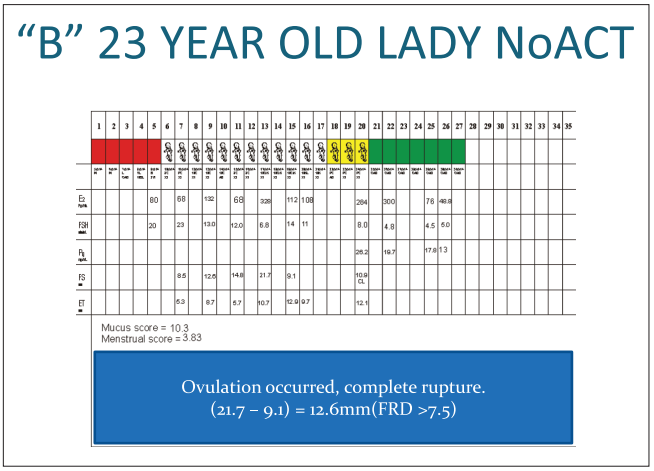

Figure 11: This shows woman "B" in this cycle no artemisinin-based combination therapy was used. She completely ruptured matured follicle around the 15th day of cycle. Regular mucus score of 10.3. Periovulatory estrogen is within normal range. Furthermore, the postovulatory estrogen and progesterone are within normal ranges

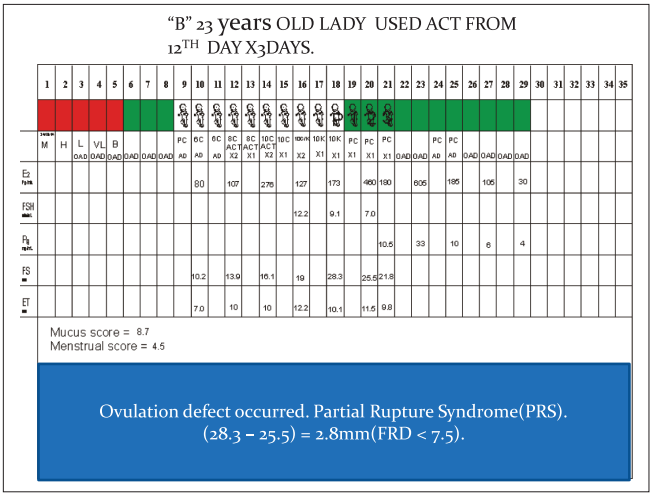

Figure 12: This shows woman "B" when she used artemisinin-based combination therapy from the 12th day of cycle. The mucus dropped to intermediate regular 8.7. A Partial rupture syndrome ovulation defect occurred. The periovulatory estrogen is within normal range, but the postovulatory estrogen is significantly high with a corresponding significant reduction of postovulatory progesterone. The E2/Pg ratio in luteal phase is high and statistically significant ($P = 0.0144$)

follicular phase, the expectation was that the mucus production will increase, but the reverse is the case. This shows that the cervix under the influence of ACT was not responsive to the apparent increased follicular phase estrogen. The possibility is that the cervical estrogen receptors have been blocked or downregulated (depleted); see Figure 21. Further investigation is needed to explain the mechanism of ACT-induced cervical mucus reduction. In Figure 22, we compared the FRD between no ACT cycle and ACT cycle and $P = 0.002589$ highly significant. In NPT, a matured follicle (18–26 mm) is considered to have fully ruptured if the FRD is ≥7.5 mm. Therefore, the revelation from Figure 22 shows that ACT

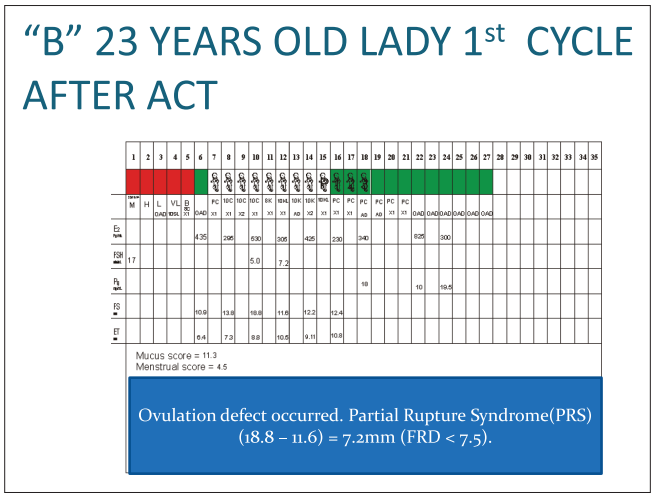

Figure 13: This shows woman "B" in her first cycle after artemisinin-based combination therapy. The mucus score is improved to regular 11.3. There is ovulation defect partial rupture syndrome prone to miscarriages. High periovulatory estrogen and postovulatory estrogen. This data are not complete, but the E2/Pg ratio at Peak + 3, Peak + 7, and Peak + 9 are high compared to normal but not statistically significant ($P = 0.149$)

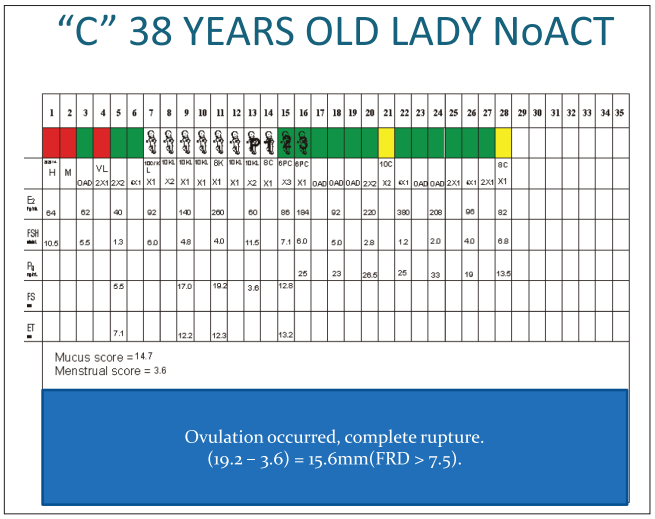

Figure 14: This shows woman "C" in cycle of no artemisinin-based combination therapy. The mucus score is regular 14.7. She completely ruptured matured follicle around 13th day of cycle. periovulatory estrogen within normal range. Both postovulatory estrogen and progesterone are high giving a normal E2/Pg luteal phase values

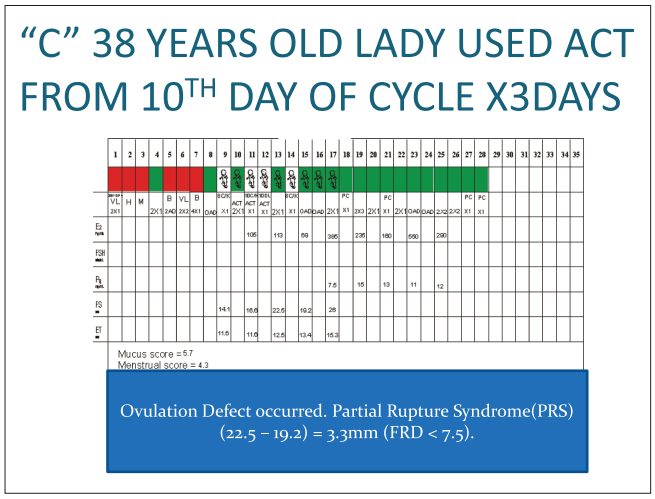

Figure 15: This shows woman "C" in the cycle of artemisinin-based combination therapy use from 10th day. Mucus dropped to limited mucus score 5.7. Ovulation defect partial rupture syndrome occurred. The E2/Pg ratio in luteal phase is high and statistically significant ($P = 0.030$) indicating a luteal phase defect

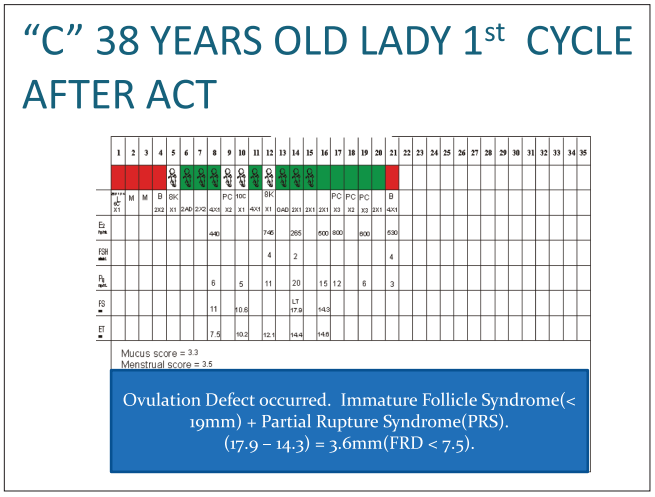

Figure 16: This shows woman "C" in the first cycle after artemisinin-based combination therapy use. The mucus dropped to limited mucus score 3.3. She developed immature follicle syndrome, with associated partial rupture syndrome. The data are not complete but the E2/Pg ratio at Peak + 5, Peak + 7, and Peak + 9 is high and statistically significant ($P = 0.041$) indicating defect of luteal phase

used in follicular phase strongly inhibited the mechanism of complete rupturing of matured follicles. This inhibition could be due to continuous activation of aromatase enzyme within the follicle or possibly that the ACT achieves this effect by causing continuous increasing LH.[20] It will be desirable to further investigate the LH level in follicular phase before and after ACT use.

Comparing the luteal phase in Figure 23, the normal with no ACT cycle, sum of three patients showed ($P = 0.305608$) (essentially normal). However, when the same women used ACT in follicular phase, their luteal phase compared to normal showed ($P = 0.010513$). There was a significant reduction in progesterone production and a corresponding increase of estrogen level. Furthermore, when the no ACT cycle was compared with the ACT cycle in luteal phase ($P = 0.011202$), again supporting that luteal phase is defective in ACT cycle. In Figure 24, the luteal phase study of the 38 years old woman showed significant defect with P values ACT cycle (0.024415), first cycle after ACT (0.034042), and second cycle after ACT(0.00421), despite the recovery of the mucus score to 14.7, the ovulation was still in defect showing partial rupture syndrome (PRS). In NPT, PRS is associated with patients with previous miscarriages.[21]

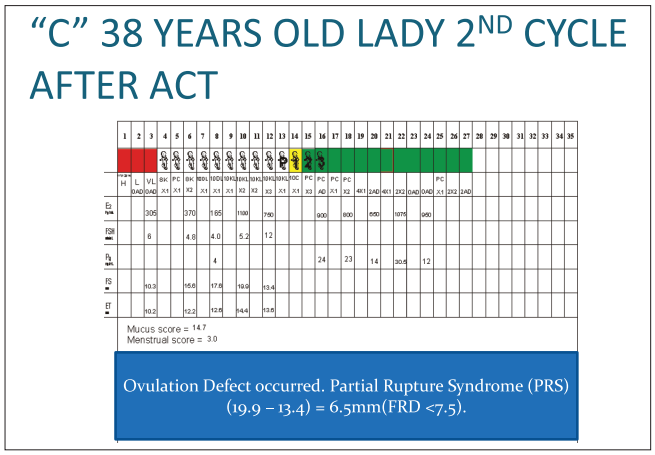

Figure 17: This shows woman "C" in the second cycle after artemisinin-based combination therapy. Mucus recovered to regular score 14.7. Ovulation defect partial rupture syndrome still noted. The E2/Pg ratio in the luteal phase is high and statistically significant ($P = 0.0042$) indicating luteal phase defect. Notice that artemisinin-based combination therapy adverse effect appears to be more in the older woman

Conclusion

The continuous search for emerging and "unsuspecting" causes of infertility is very critical if we must continue to give effective care to the increasing number of couples with infertility challenges including recurrent miscarriages. This study has shown that a very common antimalaria agent ACT (artemether + lumefantrine) has significant fertility damaging effect. The good news, however, is that this ACT effect seems to be reversible if the users of these medications are aware to discontinue. However, if ACT is continuously abused as we have now then the massive infertility effect that will follow will be best imagined. In summary, ACT artemether + lumefantrine used in follicular phase of a woman's cycle could result to the under listed conditions:

1. Causes ovulation defect PRS in the very cycle of use. Note, PRS is associated with infertility and abnormal pregnancies [Figures 9-18]
2. Causes ovulation defect in the very next cycle after use namely, PRS [Figures 9-18]

	pg/ml				mm	pg/ml	ng/ml	pg/ml	ng/ml	pg/ml	ng/ml	pg/ml	ng/ml	pg/ml	ng/ml				
Table 1	E – 4	E – 2	Peak E_2	E + 2	FS M R	P + 3 E_2	P + 3 PG	P + 5 E_2	P +5 PG	P + 7 E_2	P + 7 PG	P + 9 E_2	P + 9 PG	P + 11 E_2	P + 11 PG	P + 7 E_2/PG	SUM E_2/PG	MS	PPP
A 23 Yrs No ACT	157	265	335	85	24.9 (15.7) 9.2	285	40	265	56	220	31	525	12	315	8	7.1	10.9	12.7	10
A 11th Day + ACT P –9	420	300	620	350	27.9 (2.4)PRS 25.5; 25.8(2.5)23.3	850	23	900	15	330	10	625	4	750	3	35	62.8	8.3	6
B 23 yrs No ACT	132	68	328	112	21.7 (12.6) 9.1	284	26.2	300	19.7	76	17.8	48.8	13	56	12.2	4.3	8.6	10.3	10
B 12th Day + ACT P - 5	127	173	460	180	28.3 (2.8)PRS 25.5	180	10.5	605	33	185	10	105	6	30	4	118.5	17.4	8.6	11
B 1 Cycle after ACT	435	295	530	305	18.8 (7.2)PRS 11.6	340	18	825	10	300	19.5	No data	No data	No data	No data	15.4	No data	11.3	12
C 38 yrs No ACT	92	140	260	60	19.2 (15..6) CR3.6	92	25	220	26.5	380	27	208	33	96	19	14.1	7.6	14.7	15
C 10th day + ACT P -3	No data	105	113	69	22.5 (3.3)PRS 19.2	385	7.5	235	15	160	13	550	11	290	12	12.3	27.7	5.7	14
C 1st Cycle after ACT	440		745	265	17.9 (3.6)PRS 14.3	265	20	500	15	840	12	600	6	530	3	70	48.8	3.3	9
C 2nd Cycle after ACT	370	165	1100	750	19.9 (6.5)PRS 13.4	900	24	800	23	650	14	1075	30.5	950	12	46.4	42.3	14.7	14
Standard	88 (67-109)	(117-179) 148	(201-319) 260	(62-194) 128	FRD ≥ 7.5mm Ovulation	(53-123) 88	9.4 (5.3 – 13.5	(64-152) 108	14.4 (9.7-19.1)	(73-167) 120	15.7 (10-21.4)	(73-167) 120	13.6 (8.3-18.9)	(41-155) 98	8.1 (3.1 – 13.1)	(7.3-8.3) 7.6	(7.6-9.4) 8.7	Regular	
Sum B4 ACT	127	158	38	86	21.9 (14.6) 7.3	220.3	30.4	261.7	34.1	225.3	25.3	260.6	19.3	155.7	13.1	8.9	9.2	12.6	11.6
Sum after ACT	Incomplete	192.7	397.7	199.7	25.5 LUF (2.6) 22.9	471.7	13.7	580	21	225	11	426.7	7	326.7	6.3	20.5	35.4	7.5	10.3

Figure 18: All data including the follicular sizes; estrogen in follicular phase; progesterone and estrogen in luteal phase; the sum of three patients; and the E2/Pg ratio

FOLLICULAR PHASE STUDY SUM OF 3 PATIENTS COMPARING NO ACT VS ACT
CYCLE WITH NORMAL WITH
NO significance all p values > 0.05 NORMAL

S/N	NO ACT	NORMAL	ACT
E-2	158	148	192.7
PEAK E2	308	260	397.7
E+2	86	128	199.7
TOTAL	552	536	790.1
MEAN	184	178.6667	263.3667
STD DEV	113.2608	71.14305	116.3887
P VALUE	0.47442		0.177102
P VALUE OF NO ACT VS ACT			0.222498

Figure 19: The follicular phase estrogen did not show statistical significance in no artemisinin-based combination therapy and artemisinin-based combination therapy cycles. It "appeared" all was going fine. However, in same cycle of artemisinin-based combination therapy, mucus score has dropped! Check Figure 21

LUTEAL PHASE STUDY IN 23 YEAR OLD LADY CASE B COMPARING THE E2/Pg RATIO

S/N	NO ACT	B + ACT	1ST CYCLE
P+3	10.84	17.14	18.88
P+5	15.23	18.33	82.5
P+7	4.27	18.5	15.38
P+9	3.75	17.5	17.86
P+11	4.59	7.5	27.5
TOTAL	38.68	78.97	162.12
MEAN	7.736	15.794	32.424
STD DEV	5.089045	4.670822	28.36327
p value		0.015698	0.061793
P < 0.05 IS SIGNIFICANT			
MS	10.3	8.6	11.3
OV status	CR	PRS	PRS
MAT S	21.7	25.5	18.8
RUP S	9.1	21.8	11.6
FRD	12.6	3.7	7.2

Figure 21: There is evidence that, with artemisinin-based combination therapy, the mucus is reduced and ovulation is also defective. After the artemisinin-based combination therapy, mucus recovers first before ovulation. This suggests that mucus inhibition could be by receptor depletion while ovulation effect is enzymatic. The increased luteal estrogen production is suggestive

USING IS UNIT FOR CONVERSION E2 Pg/ml X 3.671 = VALUE TO Pmol.

PROGESTERONE ng/ml X 3.18 X 1000 = VALUE TO Pmol.

E2/Pg RATIO LUTEAL PHASE STUDY SUM OF 3 PATIENTS

S/N	NORMAL	NO ACT	ACT
P+3	0.0108	0.0084	0.038
P+5	0.0087	0.0089	0.0319
P+7	0.0088	0.0103	0.0236
P+9	0.0102	0.0156	0.0704
P+11	0.0139	0.0137	0.0709
SUM	0.0524	0.0569	0.2348
MEAN	0.01048	0.01138	0.04696
STD DEV	0.002114	0.003138	0.022222
p value		0.305608	0.010513
p value			0.011202

Significant reduction in luteal phase progesterone after the ACT use. And also a significant increase in Estrogen after the ACT use. The normal and the patients during no ACT are essentially same no significant change suggestiing the patients were of normal fertility.

Figure 23: Comparing the normal and the no artemisinin-based combination therapy cycle shows essentially a normal luteal phase status for the group of three women studied. However, after artemisinin-based combination therapy, the luteal phase was significantly defective ($P = 0.010513$)

MUCUS SCORE REDUCTION

S/NO.	NO ACT	ACT
A	12.7	8.3
B	10.3	8.6
C	14.7	5.7
TOTAL	37.7	22.6
MEAN	12.57	7.53
STD DEV	2.21	1.59
p value	0.019	

p value= 0.019 is less than 0.05.

Figure 20: Artemisinin-based combination therapy significantly reduced cervical mucus production ($P = 0.019$). This has implication for natural sperm survival in the female reproductive tract

Figure 22: Calculating the follicular rupture difference for each woman. Woman "A": No artemisinin-based combination therapy (24.9–9.2=15.7); artemisinin-based combination therapy (27.9–25.5=2.4; 25.8–23.3=2.5,average of them=(2.4+2.5)/2=2.45). Woman "B": No artemisinin-based combination therapy (21.7–9.1=12.6); artemisinin-based combination therapy (28.3–25.5=2.8). Woman "C": No artemisinin-based combination therapy (19.2–3.6=15.6); artemisinin-based combination therapy (22.5–19.2=3.3). The disruption of ovulation by artemisinin-based combination therapy from complete rupture to partial rupture syndrome is highly significant as we can see from this analysis below ($P < 0.05$)

	Studying follicular rupture differences between No ACT cycles and ACT cycles		
	No ACT	**ACT**	**Woman**
-	15.7	2.45	"A"
-	12.6	2.8	"B"
-	15.6	3.3	"C"
Sum	43.9	8.55	
Mean	14.63333	2.85	
SD	1.761628	0.4272	
P	0.002589		

SD: Standard deviation, ACT: Artemisinin-based combination therapy

3. Probably may take two cycles to recover from induced ovulation defects
4. Causes very significant reduction in cervical mucus production in the very cycle of use about 40% reduction ($P < 0.05$). Note, inadequate cervical mucus is detrimental to sperm survival [Figures 19, 20, 25]
5. Takes about one to two cycles to recover from induced cervical mucus reduction [Figures 9-18]
6. Causes very significant ($P < 0.05$) reduction of luteal phase progesterone production; suggesting an adverse effect on

the corpus luteum function (luteal phase defect), in the very cycle of use [Figure 26]

7. Causes very significant increasing remnant follicle effect, producing very high amount of estrogen in the luteal phase ($P < 0.05$) in the very cycle of use. Note, high luteal estrogen in disproportion with low luteal phase progesterone compromises implantation window; see Figures 26, 27[18]
8. The adverse fertility effect of ACT on the older woman was significant and recovery was also more delayed. See Figure 24.

Luteal phase study in the 38 years old woman comparing the E2/Pg ratio

S/N	NO ACT	C+ ACT	1ST AFTER ACT	2ND CYCLE
P+3	3.68	51.33	13.25	37.5
P+5	8.3	15.67	33.33	34.78
P+7	14.1	12.31	70	46.43
P+9	6.3	50	100	35.25
P+11	5.05	24.17	176.67	79.17
TOTAL	37.43	153.48	393.25	233.13
MEAN	7.486	30.696	78.65	46.626
STD DEV	4.069211	18.74047	64.16702	18.78946
p value		0.024415	0.034042	0.00421
p < 0.05 significant				
MS	14.7	5.7	3.3	14.7
Ov status	CR	PRS	PRS	PRS
MAT S	19.2	22.5	17.9	19.9
RUP S	3.6	19.2	14.3	13.4
FRD	15.6	3.3	3.6	6.5

Figure 24: The 38-year-old female experienced significant luteal phase defect and ovulation defects after artemisinin-based combination therapy use in follicular phase. OV = Ovulation status; MATS = Matures size; RUPS = Ruptured size; FRD = Follicular rupture difference; Cr = Complete rupture; PRS = Partial rupture syndrome

LUTEAL PHASE STUDY E2
AND Pg VALUES
COMPARED IN NORMAL,
NO ACT AND ACT CYCLES.
Computed in Pmol/l

	E2			Pg		
S/N	NORMAL	NO ACT	ACT	NORMAL	NO ACT	ACT
P+3	323.048	808.721	1731.611	29892	96672	45566
P+5	396.468	960.701	2129.18	45792	108438	66780
P+7	440.52	827.076	825.975	49926	80454	34980
P+9	440.52	956.666	1566.416	43248	61374	22260
P+11	359.758	571.575	1419.576	25758	41658	20034
SUM	1960.314	4124.739	7672.757	194616	388596	189620
MEAN	392.0628	824.9478	1534.551	38923.2	77719.2	37924
STD DEV	51.28901	158.3068	476.6369	10509.89	26823.07	19138.63
p value		0.001189	0.002798		0.014139	0.46086
p value			0.013015			0.01484

There a significant increase of Luteal phase E2 in ACT cycle compared to Normal and NO ACT cycle, but there was no
significant decrease of Progesterone between the ACT cycle and Normal but there was significant Progesterone decrease

between the ACT cycle and the NO ACT cycle. The result is net E2 dorminance in the ACT cycle.
There is significant increase of E2 in NO ACT cycle compared to Normal and also a corresponding increased significance

of Progesterone in the NO ACT cycle compared to Normal. The result is a close to normal E2/Pg ratio.

Figure 26: There is significant increase in luteal estrogen in artemisinin-based combination therapy cycle compared to norma, ($P = 0.001189$). There is a significant luteal progesterone increase in no artemisinin-based combination therapy cycle compared to normal ($P = 0.014139$) (The net effect is a normal luteal function). There is a significant increase in luteal estrogen in artemisinin-based combination therapy cycle compared to no artemisinin-based combination therapy cycle ($P = 0.013015$). There is a significant luteal progesterone decrease in the artemisinin-based combination therapy cycle compared to the no artemisinin-based combination therapy cycle ($P = 0.01484$) (the net effect is a defective luteal function)

We would have followed up these women to determine when they will completely regain their pre-ACT fertility status, but the lack of fund was a major challenge.

We believe that this study calls for a more critical look into the current use of ACT in all women, especially of this possibility of fertility compromise, of which many people who use ACT indiscriminately and frequently are not aware about.

MUCUS SCORE REDUCTION

	A	B	C	SUM	AVERAGE ÷ 3
NO ACT	12.7	10.3	14.7	37.7	12.6
ACT USED	8.3	8.6	5.7	22.6	7.5
% REDUCTION	35	17	61	40	40

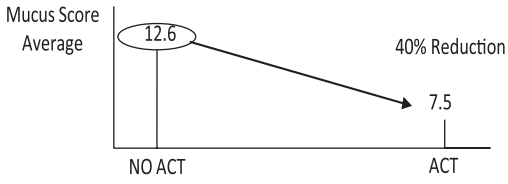

Figure 25: A sum total of 40% cervical mucus reduction between cycles of no artemisinin-based combination therapy and cycle of artemisinin-based combination therapy use. From regular mucus score of 12.6 to intermediate limited mucus of 7.5

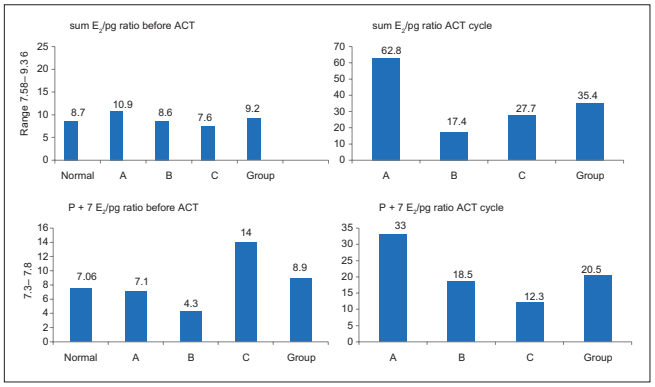

Figure 27: The sum of E2/Pg ratio is more informative like giving the integrated value of luteal estrogen and progesterone. We noticed that the E2/Pg ratio at Peak + 7 in woman C was higher before artemisinin-based combination therapy than after artemisinin-based combination therapy (this was due to inadequate sample collected on that day)

Recommendation(s)

The CrMS and NPT the tool and technique that were employed to study the effect of ACT on fertility are a unique concept that holds enormous opportunities for family physicians, gynecologist, and all fertility care health workers. We encourage all interested to seek this knowledge for the general well-being of our patients and also for an improvement in the health care services in the country. Specifically for the management of infertility special care should be taken concerning the indiscriminate use of ACT. We recommend that all women in reproductive age seeking to use ACT for malaria treatment are informed that ACT also has reversible contraceptive effects.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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