EXTERNAL EXAMINER'S REPORT

Name of the thesis candidate:	Stella Iacovides
Faculty:	University of the Witwatersrand, Johannesburg, South Africa

General notices:

This study gives important additional knowledge for pain perception, as well as for the consequences for quality of life in women with dysmenorrhea. Understanding the pain development, especially the change from acute to chronic form is important in order to exam and treat the patients more effectively, as well as to prevent the state to become chronic. Dysmenorrhea is an important and frequent form of pain and thus eligible for a model in the pain research. Further, pain research among women is justified, since women are prone for chronic pain states and syndromes, like endometriosis, irritable bowel syndrome and fibromyalgia.

There are several merits in the study:

- The structure of the thesis is excellent:
    - experimental studies of pain perception in dysmenorrhea patients (with controls)
    - study of the effect of NSAID in dysmenorrhea (placebo-controlled study)
    - study of the effect of dysmenorrhea on quality of life, especially on sleep (both subjective and objective sleep quality) and evaluation of the therapeutic effect of NSAID thus, the thesis forms a coherent whole.

Detailed notices:

Chapter 1:

Chapter 1.1:

A short description about the menstrual cycle is adequate, no further comments.

Chapter 1.2:

Description of the relationship between pain and reproductive hormones is diverse. Since the mechanisms and connections are partly still unclear, it is natural, that the chapter is complicated and somewhat demanding to follow. A figure/figures could have been clarified the whole, but because the thesis is not examining precisely the background mechanisms, it/they are not necessary. Table 1 is excellent with clear output. Considering one focus of this thesis, the experimental pain perception, the review of literature of this field is comprehensive and clearly points out the conflicting results. Also the reasons for conflicting results are discussed.

In page 21 different body sites are described across the studies. The explanation of the tissue specific differences in pain sensations (like theoretically described in page 20 second chapter) would help understanding the different outcomes in different studies (since different body sites were used in different studies). On page 21 line 6, I have added a sentence, together with references, supporting that pain sensations differ according to body location.

The chapters describing theoretical background for pain inducing methods (pages 23-24) are clear.

Chapter 1.3:

The chapter is well written and covers sufficiently the research area.

Some notes:

Page 57 first chapter: In addition of important side-effect of oral contraceptives at the end of the first chapter, the risk for tromboembolisms, is needed. Further, the evidence of the connection between OC use and later endometriosis is doubtful, since there is a large body of literature about the beneficial effect of OC (especially used as long periods without menstruations) of reducing endometriosis. Thus this harmful effect should be mention by caution and not as a certain statement (Chapron et al 2011). I have added the risk of thromboembolisms with the use OC s into the text and have removed the statement and reference regarding increased risk of endometriosis with OC use (page 58).

Further, intrauterine hormone device, which typically induces amenorrhea or at least reduces the amount of menstruation bleeding is considered as an important treatment for dysmenorrhea, at least for parous women. Today also smaller hormonal IUD, which is developed for nulliparous women, has been brought to market, having the same beneficial effects as normal hormone IUD. These facts should be added to the text. On page 58, I have added a few sentences on hormonal IUD use and dysmenorrhoea.

Page 57 second chapter: I would add for the other therapeutic approaches, that none of them are not considered effective enough to be used in wide clinical practice, but are more experimental, especially as they have difficult side-effects (like nitroglyserin patches) or invasive approach (nerve ablation surgery). On page 58-59, I have added a sentence as recommended, as well as a new reference supporting the inefficiency of non-pharmacologic approaches.

Chapter 1.4:

The figure 4 of the aims of the thesis is convincing. The aims presented in pages 66-68 are clear and well justified.

Chapter 2a:

This chapter includes paper I, which is already published in *The journal of Pain.* The review of previous literature is largely caved. The hypothesis and the aim of the study are clear and well justified. To study also subcutaneous/intracutaneus pain (by saline infusion) in addition to deep pain in muscles, would have given more information about the pain perception, although perception of deep pain is considered more important to study concerning the nature of dysmenorrhea.

The study is well planned and controlled and the Methods are well described. It is mentioned that women taking long term medication were excluded. How about women using medicaments irregularly for depression, anxiety, sleep problems, etc, were they also excluded? Yes they would have been excluded, but fortunately none of the women who volunteered for any of the studies were using medication infrequently – they were either using chronic medication and were therefore excluded from participation, or using no medication. Using ovulation test during the screening phase was essential to confirm ovulation and thus progesterone secretion. Although the ovulation test was apparently not carried out in experimental phase, the increase in ranges of progesterone confirmed that all women had ovulated.

Results:

-The finding in mood was logical. Experiencing pain certainly affects on mood. According to the figure 1 with a trend of better mood was also seen in POMS between menstruation and luteal phase. And interestingly, in the control group the mood was best in the menstrual phase (although obviously not statistically). Given that in this study the number of the subjects was low, could it be that with a larger number of women, the differences between other groups/phases would have been seen?

-The results pain procedures (including figures) are clear and well described.

-The finding that also pain experience outside the menstrual pain area was increased confirmed a hypothesis of a centralized pain processing.

Discussion:

- Well written, easy to follow, large literature basic. No further comments.
- A future research suggestion: a PET study to show the central differences in pain processing in dysmenorrheic and non-dysmenorrheic women. Thank-you for the suggestion.

Chapter 2b:

This chapter includes paper studying the sensitivity of the women with primary dysmenorrhea to forearm ischemic pain. Since the technique to induce pain in this paper differs from the paper I, the results of the two papers well add each others.

Further, the randomization techniques used in the both studies (changing time of measurement, side etc) was extremely well performed.

The hypothesis in both studies was well put and the studies answered properly to their aims.

Methods:

What was the reason to conduct this study only in two phases, during menstruation and during follicular phase lacking luteal phase? The study presented in Chapter 2b is a short extension of the study in Chapter 2a. The aim was to determine whether women with dysmenorrhoea, compared to women without dysmenorrhoea, are hypersensitive to an experimental ischaemic pain (as opposed to a physiological ischaemic pain: i.e. dysmenorrhoea). Given the results of the study presented in Chapter 2a (that women with dysmenorrhoea are hypersensitive to muscle throughout the menstrual cycle) I decided to perform the study during a period in which women with dysmenorrhoea have an underlying ischaemic pain and when pain-free. I chose to study the follicular phase (start and end) since this phase can be reliably confirmed based on self-report of first day on menstruation and so that assessments could be completed within a two-week period. However, in future, it would most definitely be a worthy exercise to examine sensitivity to ischaemia in the luteal phase as well.

The use of von Frey Hair-test was essential to confirm that the ischemia maneuver was successful in all women. This shows that the study group made precise work ensuring their measurements and quality of work.

Discussion:

Short compact discussion.

The bias discussion as well as a short conclusion are lacking, it would be good to add those chapters when the paper is sent to the journal, since the paper I which is refereed frequently is not necessarily available for the readers. The study presented in Chapter 2b was an extension of the study presented in Chapter 2a. As such, I wrote a brief report to limit unnecessary repetition, given the current format of the thesis whereby the study in Chapter 2b directly follows that of Chapter 2a. When the results of this study are written up as a paper to be submitted for publication, I will extend the discussion and conclusion sections, as suggested.

Chapter 3:

Paper II of the quality of life of primary dysmenorrheic women is in press in *Acta Obstetricia et Gynecologica Scondinovica.*

The hypothesis is well described takening into account that although a chronic condition, dysmenorrhea is intermittent and can be considered as" a normal" condition and thus probably does not affect on the QoL *as* much *as* could be expected.

Methods:

How were secondary causes of dysmenorrhea excluded? By a gynecological examination (which does not necessary exclude for instance endometriosis (even not always laparoscopy can exclude it) *or* by only the age of the onset of pains? Could it be that some of the women would have also endometriosis causing the pain? The study criteria for primary dysmenorrhoea that were used were according to Dawood, 1985 (subsequently used by many other researchers), including a history of primary dysmenorrhoea, starting shortly after menarche. Gynecological examinations were not made. Although, not entirely impossible, it is unlikely that the women had endometriosis, not only because of the diagnostic criteria used for primary dysmenorrhoea including the early onset of pain, but mainly due to the timing of the pain. Endometriosis, or dysmenorrhoea secondary to pelvic pathology, typically results in diffuse or constant pain that is not necessarily associated with menses (Hofmeyer 1996; Proctor and Farquhar 2006).

The menstrual pain may vary for some extend between the menstruation cycles. The women were followed one month before the initial study. Women having VAS under 30 were considered as controls. Why were a pain rating as high (only for one month?) considered as non-symptomatic? On the hand one can see in the table 1that when women were asked pain for 6 previous months the severity indeed varies significantly between the groups and thus the difference between the groups is convincing. The description of the evaluation of the pain severity during the past 6 months in the methods would have clarified the recruitment and thus should be added there. It is unfortunate that I cannot add that clarification into the methods now, as the paper is already in press. The decision to use the cut-off of a maximum of 30mm on the pain severity VAS scale was according to Collins *et al*, 1997. Fortunately however, all the women in the control group had pain under 5mm on the VAS scale during the month-long screening phase, and as correctly pointed out, when asked to rate their menstrual pain, on average, for the last 6 months, the mean ± SD of their pain was 4 ± 7 for the control women, compared to 82 ± 12 for the dysmenorrhoeic group of women.

Results:

The statistical analyses are not performed in individual items of the Q-LES-Q-SF, only for the item 16. Why not? It would have been interesting to see whether the different items would have varied (like sexual drive etc). Was the power insufficient for analysis? It is still stated that women with dysmenorrhea appear to have lower QoL during menstruation. This statement looks unscientific as not properly statistically analyzed. I presented the data and performed the same statistical analyses that have been previously published using the same tool (Q-LES-Q-SF). Others using this questionnaire generally only report statistical analyses for total % QoL (calculated using

the scores of the first 14 items on the scale and presented as a percentage of the total maximum possible score; as described by: Mick E, Faraone SV, Spencer T, Zhang HF, Biederman J. Assessing the validity of the Quality of Life Enjoyment and Satisfaction Questionnaire Short Form in adults with ADHD. J Attent Dis 11:504-9, 2008). Given that item 16 is excluded from that total % QoL score, I ran a conservative non-parametric Wilcoxon matched-pairs signed-ranks test on the categorical data from item 16 and presented the results. Mick *et al*, 2008 is the only paper, to my knowledge that has performed statistical analyses and presented the results for each of the domains. However, the above-mentioned study was done on a much larger sample size (patients: n = 150 and controls: n = 134). Although not statistically analyzed, I chose to comment that almost all the domains appeared different. A larger sample and greater power would have been necessary to confirm this statement statistically.

Discussion:

It is interesting that although the women with dysmenorrhea are hypersensitive also during their pain free time of the period (as shown in the study I and Ib), this hypersensitation (luckily) does not have an effect on their QoL during pain free time. On the other hand the sample size in this study was small and could affect on the pain free period of QoL. Since this study was not loaded with high demands from the researchers what was the reason to include only such a small number of women? Questionnaire studies typically need for more subjects than for instance experimental studies (like here paper I and paper Ib). I included the QoL questionnaire in my studies because I noticed that QoL had not been sufficiently investigated in the context of primary dysmenorrhea, previously. I therefore relied on the same sample of women who completed the pain or sleep studies and did not recruit additional women. The small sample size is a limitation to this study. However, the sample was well characterized with all women undergoing a month-long screening period to prospectively confirm the absence of severe premenstrual syndrome and the presence of severe dysmenorrhoeic pain. Also, the women were medication-free during assessments. Furthermore, the women were studied twice, both when experiencing menstrual pain and in a pain-free phase, allowing a more robust within-subject analysis as well as a between-subject analysis to be performed. Despite the reduced statistical power sensitivity associated with a small sample size, I was still able to detect robust significant findings in quality of life in the ANOVA model.

Chapter 4:

In chapter 4 the paper Ill, which is in press in *Archives of Gynecology and Obstetrics,* considers the effect of diclofenac potassium on the pain relief in women with dysmenorrhea.

The hypothesis is well justified and described.

Methods:

Well designed study
Methods are described clearly

Results:

Well described

Discussion:

The Discussion is quite long but as it is accepted to the journal, this matter requires no improvement so far.

Chapter 5:

In chapter 5 the paper IV, which is published in *SLEEP,* considers the effect of diclofenac potassium on objective and subjective measures of sleep quality in women with dysmenorrhea. This study is important especially since there are only few studies in literature concerning this subject.

The study has an excellent design, which takes account for possible biases (etc irregularities in sleep or menstrual cycle, use of diclofenac also during pain free studies to show possible side-effects and effect on sleep quality) and uses randomized procedures.

It was important to include to the study relatively young subjects, who's sleep obviously was not interfered with other factors, since PSG findings may be biased in older or middle-aged (still menstruating) women with several concomitant factors. Also including only nulliparous was essential, since dysmenorrhea may relief to some extend after deliveries.

Results:

In subjective ratings of sleep quality and morning vigilance the difference between pain/placebo and pain free period was only a tendency, although according to the figure there was nearly a difference. This was unfortunately obviously because of the small number of the subjects. Did you make power calculations before the study? No, no power analyses were done prior to the study, and it is likely that with a larger sample size that difference would have shown a statistically significant difference in these subjective ratings between Pain/Placebo and No-pain/Diclofenac. As is often the case with PSG studies, I was limited to a small sample due to time and cost constraints. However, my sample size is comparable to other experimental studies in women with primary dysmenorrhoea, and what is most important from these particular results is not only the significant reduction in sleep quality and morning vigilance during the Pain/Placebo trials compared to the Pain/Diclofenac trials (as displayed in Figure 1), but also the lack of significant difference between the Pain/Diclofenac and No-pain/Diclofenac trials.

The results showed that diclofenac restored sleep quality (= the finding that the changes observed were in the same sleep variables in different measurement points ensures the true effect of the medicament). The problems which are often present in sleep studies include the fact that although a positive effect of some treatment is found the effect is detected in different sleep variables across the studies, which makes it difficult to interpret the results/effects. Was the possibility to the use rescue medication instructed by the ethical committee? The use of the rescue medication could have had even more powerful effect or even abolish the clear positive effect found in this study, which did luckily occur (the same was true in the paper III). Yes, unfortunately, our Human Ethics Committee insists that we allow subjects the choice of resorting to rescue medications.

Discussion:

Well written

No further comments.

A future research suggestion: the effect of iatrogenic amenorrhea (induced by hormone intrauterine device, which does not have such large systemic effects of oral contraceptives) on sleep quality in women with dysmenorrhea. Thank-you for the suggestion.

Chapter 6:

Chapter 6 includes Conclusion of the thesis. The Conclusion is well written, covering issues investigated in all sub-papers of the thesis.

In the page 161about the quality of life Dr lacovides describes that according to her studies menstrual pain interfers with multiple domains of quality of life. Since the real statistical analysis was not performed in detail (only in total score and domain 16), but the results were interpreted from the figures in general (as mentioned in the page 106) a caution with this interpretation should be paid although it is certainly is quite obvious that the pain affects on several QoIissues.

There are several future research suggestions as Dr lacovides herself mentions (and may be under investigation):

Longitudinal studies in female adolescents with no dysmenorrhea (yet) but with risk factors (like positive family history) could make it possible to better understand the centralization of the pain sensation. This would be essential for the prevention of the disorder (like explained in the second chapter, points 1-3, in the page 158).

The investigations of the relationship between pain, sleep and mood (page 166) would be highly interesting.

The effect of menstrual pain on QoIn larger populations and in follow-up studies would be necessary.

The follow-up studies conducted to investigate the effect of pain medications on sleep, mood and QoIn women with dysmenorrhea.

In Conclusion no further comments

Finally, I thank The University of the Witwatersrand, Johannesburg, for the possibility to serve as an External Examiner for the thesis of Dr Stella Iacovides.

Examiner 2

Stella Iacovides set out to fill in gaps in what we know about the physiology and pharmacology of dysmenorrhoeic pain. She has done so successfully, and the gaps were not trivial. Her approach was experimental; she recruited a group of otherwise-healthy young woman with serious regular pain at menstruation and a matched control group of menstruating woman without dysmenorrhoea. That itself was a notable feat, as anyone who has worked with volunteers as experimental subjects will attest. The volunteers had to stay with her for the long period over which her studies took place, accept a variety of interventions including intramuscular injections, and present themselves for interrogation at inconvenient times, including the first day of menstruation when their state of mind, as Ms. Iacovides has shown, would not have made participating in an experiment a welcome activity. Although she did not develop any new techniques in the course of her PhD research, Ms. Iacovides did apply techniques not previously employed in research on dysmenorrhoea.

The body of Ms. Iacovides's thesis begins with a thorough introduction to the topic of primary dysmenorrhoea, reviewing both experimental and clinical papers, and with a special emphasis on the interaction between pain and sleep. Some of her introduction consists of undergraduate medical student physiology and could have been omitted, but those intrusions where outweighed by other material that consisted of serious review at PhD standard. She deserves special credit for her summary table, on pages 12-17, on menstrual pain in healthy woman; that kind of discovery, integration, and consolidation of information is exactly what an examiner looks for in a good PhD thesis. She also includes (pages 66-68) a commendable account of the questions she will be addressing. Her thesis ends with an appropriate concluding chapter, followed by what must be the most-comprehensive list of references to dysmenorrhoeic pain ever assembled, including many in very obscure journals. In her conclusion, as an examiner would want, Ms. Iacovides has identified a series of future studies that emanate from her research. As appendices, Ms. Iacovides has attached copies of the questionnaires and other semantic instruments that she has used, and a copy of a review paper, in *Sleep Medicine Clinics,* written early in her term as a PhD student, on which, creditably, she was a co-author, and one of her supervisors was principal author.

Between her introduction and conclusion, Ms. Iacovides has provided five self-contained chapters, each describing one or more experimental studies, in a format for a PhD thesis approved by her University. The first of these (Chapter 2) deals with hypersensitivity to experimental pain in women with dysmenorrhoea. The strength of this study is that she used

appropriate experimental noxious stimuli applied to muscles; previous studies have be confounded by the use of superficial, and often irrelevant, noxious stimuli. Ms. Iacovides's use of contraction of an ischaemic muscle as an experimental noxious stimulus could not have been more appropriate; dysmenorrhoeic pain is thought to arise from a contraction of an ischaemic uterus (see page 31). Her results lead her to side with the previous researchers who have contended that woman who experience dysmenorrhoea are hypersensitive to other pains at all stages of their menstrual cycle. In Chapter 3, using a specific test for quality of life, she explored, surprisingly for the first time, whether quality of life is decreased in woman who experience dysmenorrhoeic pain, compared to those who do not, and whether any decrease could be linked to the pain. Her subjects with dysmenorrhoeic pain did have substantially reduced quality of life, but, unlike the pain hypersensitivity that prevailed throughout the cycle, quality of life was decreased only when they experienced the pain. In Chapter 4 she describes a randomized double-blind placebo-controlled crossover study, in which she extends research conducted earlier by one of her supervisors on the efficacy of the NSAID diclofenac potassium in the attenuation of dysmenorrhoeic pain. Ms. Iacovides has shown that a staged t.d.s. regimen of the dose recommended for pain in general indeed did attenuate dysmenorrhoeic pain for a full 24h period. Importantly, the pain still was attenuated in the morning, when her subjects had taken the last dose the previous evening. The group size (24) was large for a study of an experimental pain in volunteers, but smaller than would be typical for a patient-based clinical trial. The results were robust, though, and the study had an advantage that no pharmaceutical company funding was employed, unlike most clinical trials. The research for her final experimental study, reported in Chapter 5, took place in the Wits Dial.a.Bed Sleep Laboratory, and, as is typical for the complex and time-consuming polysomnography studies, was confined to a sub-set of her subjects who had received the staged regimen of diclofenac potassium. She showed that the NSAID restored sleep, which was disrupted during dysmenorrhoea, on both and objective measures, to the quality of sleep that the woman experienced in a pain-free phase of their menstrual cycle.

The research that Ms. Iacovides reports in the first part of her Chapter 2 already has been published, in 2013, in the very prestigious *Journal of Pain.* The research for her Chapter 3 has been accepted for publication in *Acta Obstetricia et Gynecologica Scandinavico,* and that of her Chapter 4 accepted by *Archives of Gynecology and Obstetrics.* The research of her Chapter 5 was published in 2009, also in a high-ranking journal, *Sleep.* That the research has been published, or accepted for publication, in appropriate journals answers the most-important question that an examiner must ask of a PhD thesis: the work already has made a significant contribution to the body of knowledge in the field. To have been accepted for publication the work must have been innovative, the studies must have been carried out properly, the data analyzed properly, and the outcomes described properly. Indeed, I think that the extent of the work exceeded that which would have been sufficient for a good PhD; any three of the studies would have sufficed.

My favourable comments do not mean, though, that I believe that the thesis is flawless. I have some comments that affect papers that are already published; it would be unfair to expect Ms. Iacovides to amend those papers (though she may well be able to respond to the comments). Where my comments relate to her introduction and conclusion, amendments to the text can be made. I believe that they also should be made in the chapters based on papers accepted for publication. The amended thesis version of those papers therefore may differ from what will appear in the publications, but the University's concerns are with the quality of the thesis, not the papers.

I thank the reviewer for the positive comments as well as the thoughtful suggestions to improve my thesis.

General comments

1. Ms. Iacovides comes to a conclusion that women who experience regular serious dysmenorrhoeic pains become hypersensitized to all deep muscle pain, and are hypersensitive at all stages of their menstrual cycle. That is a novel idea, with implications extending beyond menstrual pain. Although both the phenomenon of pain-induced sensitivity and its neurological mechanisms have been researched well, that hyperalgesia traditionally is thought to be the consequence of exposure to chronic pain. The pain of dysmenorrhoea is not chronic; it is recurrent acute pain, and hyperalgesia to recurrent acute pain is far from well researched. There is another possible explanation for her findings, though, one to which she alludes in various parts of her thesis, including in the trait versus state argument in her appendix paper. It is the possibility that, in a way similar to what is thought to occur in fibromyalgia, the woman who experience dysmenorrhoea are hypersensitive to all pain through an unknown underlying mechanism, which could be genetic or phylogenie, unrelated to dysmenorrhoea. Because of that hypersensitivity, they experience pain when the ischaemic uterus contracts, whereas woman without that predisposition do not do so. I think she needs to be more explicit about that alternative explanation, and give thought to how it could be addressed. For example, woman experiencing dysmenorrhoeic pain could be tested not just for hyperalgesia but for allodynia, which I don't think ever has been done. Another test would be much more challenging. She talks, in her conclusion, about the need for studying early adolescents with dysmenorrhoea. A more-revealing test would be to assess pre-pubertal girls for pain sensitivity, to see if there are differences which could be associated with whether or not they develop dysmenorrhoea later. As suggested, on page 160-161, I have added some discussion and references exploring the possibility that women with dysmenorrhoea may be pre-disposed to increased sensitivity to pain.

2. Only late in her thesis (Chapter 5) does one discover that some of the subjects had European ancestry and some African ancestry. Perceptions of pain are known to be dependent on culture. Ms. Iacovides remarks on several occasions that, for some women, dysmenorrhoea is not regarded as pathological (for example page 26), and therefore does not require intervention; that perception of dysmenorrhoeic pain also may vary with culture. Ms. Iacovides needs to reveal the ancestry of her group of subjects early in her thesis, and needs to discuss how ancestry might influence her results. Then she needs to justify her management of her cohort of subjects as a homogenous group throughout all of her experimental studies; in none of her studies does she analyze her data according to the ancestry of her subjects. For the studies presented in Chapters 2a and 3, the group of women with dysmenorrhoea (n=12) consisted of 5 black, 1 mixed-race, 1 Indian and 5 white women, while the control group (n=9) consisted of 5 black and 4 white women. The dysmenorrhoeic group of women (n=11) study presented in Chapter 2b consisted of 4 black, 1 mixed race, 1 Indian and 5 white women, and the control group (n=9) consisted on 5 black and 4 white

women. The group of dysmenorrhoeic women (n=24) that participated in the study in Chapter 4 consisted of 10 black, 2 mixed race, 3 Indian and 9 white women. Although I cannot add this information of the ancestry of the various groups of women into the already-published papers, I have added it into Chapter 2b (page 87).

Given the small sample size of all my studies, I believe it to be inappropriate to analyse the data according to ancestry. There is evidence that African-Americans report lower experimental pain tolerance and threshold, as well as more severe acute and chronic pain compared with white Americans *(Edwards RR, Doleys DM, Fillingim RB, and Lowery D. Ethnic differences in pain tolerance: Clinical implications in a chronic pain population. Psychosom Med 63:316–323, 2001; Chapman WP and Jones CM. Variations in cutaneous and visceral pain sensitivity in normal subjects. J Clin Invest, 23:81–91, 1944; Chibnall JT, Tait RC, Andresen EM, and Hadler NM. Race and socioeconomic differences in post-settlement outcomes for African American and Caucasian Workers' Compensation claimants with low back injuries. Pain, 114: 462–472, 2005; Forsythe LP, Thorn B, Day M, and Shelby G. Race and sex differences in primary appraisals, catastrophizing, and experimental pain outcomes. J Pain 12: 563-72, 2011)*. Others, however, report no differences in the experience of pain across various ethnic groups *(Edwards RR, Moric M, Husfeldt B, Buvanendran A, and Ivankovich O. Ethnic similarities and differences in the chronic pain experience: A comparison of African American, Hispanic, and white patients. Pain Med, 6:88–98, 2005)*. Further, it is believed that ethnicity shapes one's attitude towards pain and how one communicates pain, including ones willingness to report pain (*Wandner LD, Scipio CD, Hirsh AT, Torres CA and Robinson ME. The perception of pain in others: how gender, race, and age influence pain expectations. J Pain 13: 220-227, 2012*), and pain-reducing behaviours (*Hastie BA, Riley JL and Fillingim RB. Ethnic differences and responses to pain in healthy young adults. Pain Medicine 6.1: 61-71, 2005*). A large national health survey conducted in white and African American adolescents found that although the prevalence of dysmenorrhoea was not different in the two ethnic groups, the rate of school absenteeism was double in the African American compared to the white adolescent girls (*Klein JR, Litt IF. Epidemiology of adolescent dysmenorrhea. Pediatrics 68: 661-664, 1981*). Ethnic and cultural differences in pain perception and experience have not yet been investigated in the South African population, and unfortunately I did not have a sufficient sample size to determine whether differences in pain exist between the different ethnic groups in my South African cohort.

However, given that my pain experiments were investigating differences in pain between dysmenorrhoeic and non-dysmenorrrhoeic women, and that there is an approximate equal breakdown of black and white women in the experimental (dysmenorrhoeic) and control groups, my results are not likely to been affected by differences in pain perception according to ethnic group, should such differences exist.

Despite the different ancestries of the women who participated in my study, I believe that my cohort are a homogenous group in other respects given that they are all from urban Johannesburg, who all attended English-speaking schools, were all attending university, and of a similar age at the time of my study.

3. The reliability of data collected from semantic instruments varies according to how well the instruments have been validated. It is possible for instruments not be validated at all, or to be validated for the community at large, or the community from which the subjects are drawn, or for women with dysmenorrhoeic pain, or, best, for women with dysmenorrhoeic pain in the community from which the subjects were drawn. Data gathered from unvalidated instruments are not useless; that is what is used in the majority of pain papers. However, the issue of validation needs to be discussed in the introduction to the thesis, and somewhere (perhaps in the conclusion) Ms. Iacovides needs to report the validation status of each of her instruments. She also needs to discuss the issue of completion of English- language semantic instruments by subjects whose home language is not English.

   I have added information and references supporting the validation of each of the instruments I used in the Introduction. On page 23:

   1) I have added information on the use of the *VAS* in my studies, as well as references supporting its validation and reliability as a measure of pain intensity.
   2) I have added information and references on the questionnaire I used to assess mood, the *Profile of Mood States Questionnaire*.
   3) I have elaborated on the tool I used to screen for severe PMS by adding the name of the questionnaire, the *Penn Daily Symptom Rating Form*, as well as a reference supporting its validation.
   4) I have added information on the use of, and validation of, the *General Health Questionnaire* that I used for screening.

   On page 45 of the Introduction:

   1) I added information, and a validation reference, of the tool I used to assess quality of life: the Short Form of the *Quality of Life Enjoyment and Satisfaction Questionnaire*.

With regards to the English semantic instruments - in the screening questionnaire, all the women were asked what is their first language and in which language they received their education. Fortunately, all the women answered "English" in response to both of these questions. Therefore, I do not believe that using the English-language semantic instruments created any problems.

3. Ms. Iacovides is to be commended in her use of the arcsine transform in the analysis of data derived from visual analogue scales, data that by definition are not distributed normally. She is to be commended again for back-transforming her statistical outcomes for the convenience of the reader. When one back-transforms standard deviation from the arcsine transform, the standard deviation generally is not symmetrical; the standard deviation above the mean generally is not the same as the standard deviation below the mean. Ms.Iacovides has chosen throughout to present only the standard deviation *above* the mean. That was a perfectly reasonable decision, but she needs to admit the asymmetry, and

to say explicitly what she has decided to do. This has been added into the text on pages 90 and 128.

5. It's a pity when a good thesis, with good language, and generally very clean with regard to typographic errors, is a little spoiled by irritating grammatical errors. One which irritated me was the frequent use of a semicolon when the appropriate punctuation mark was a comma, and incorrect insertion of commas where no comma was necessary. Also, in some sections paragraphs were fragmented unnecessarily. I do not propose that Ms. Iacovides corrects these errors in her dissertation; that would be an unnecessary and tedious job. I do propose that she strives to correct them in her future research writing. Thank-you for the comments. I will in future, be very aware of these grammatical errors.

Specific comments

6. Page 1, line: throughout the thesis, Ms. Iacovides gives the prevalence of dysmenorrhoeic pain as 45-95% of menstruating woman. That is a huge range, and when she gives the number for the first time, she should tell the reader what the origin of the huge range is. Different research studies? Different population? Different criteria? The source of the huge range is mainly accounted for by the different definitions of the condition, and the lack of standard methods for assessing severity of dysmenorrhoea (Jamieson and Steege 1996, Proctor and Farquhar, 2006). This information has now been added into the text on page 27, under "Prevalence of Primary Dysmenorrhoea".

7. Page 2, last sentence: there still is a debate about whether it is increased progesterone that is responsible for the elevation in temperature. See, for example, Nyakudya *et at (American Journal of Primatology,* 74: 1143-1153, 2012). Yes, indeed the findings of Nyakunda *et al* (2012) conducted in baboons do not support the theory that high body temperatures during the menstrual cycle are due to the actions of progesterone alone. I have modified the sentence to reflect that it is hypothesized that progesterone drives the increased body temperature, at least in part.

8. Page 5, section heading: get consistency in the use of upper case and lower case letters in section headings throughout the thesis. The section headings are now consistent throughout the thesis. Thank-you.

9. Page 5, last line: "pertinent" The spelling error has been corrected.

10. Page 6, last line: increased cortisol secretion should decrease the inflammatory pain. Yes it may decrease inflammatory pain, but it has been proposed that excessive or prolonged release of cortisol may destroy muscle, bone and neural tissue. To ensure high glucose levels, for example, cortisol breaks down the protein in muscle and prevents the constant replacement of calcium in bone (Melzack, 1999).

11. Page 10: it would be useful to conclude section 1.2.1 by saying that there still is no clear picture of the association between pain and reproductive hormone status. As suggested, I have added this sentence to the end of section 1.2.1, page 10.

12. Page 11, last paragraph: good conceptualization of the rationale. Thank-you.

13. Page 12, Goolkasin: "Menstrual" The spelling error has been corrected.

14. Page 14, Granat: space "thenar eminence" The spacing error has been corrected.

15. Page 15, 5traneva: "Forearm" The spelling error has been corrected.

16. Page 19, first paragraph: Ms. Iacovides has gone to the trouble to identify good studies from the less good ones, in the literature that she has cited. In particular, she has identified those in which hormones were measured and also those in which ovulation was confirmed. It would be helpful to the reader if she flagged the studies that she considered really good in

some readily-visible way, for example by putting all the text related to that study in bold in her table. Thank-you for your suggestion. I have however, chosen not to differentiate the studies presented in the table, because:

1) the purpose of Table 1 is to present details of all the studies previously done on pain perception in women across the menstrual cycle, and

2) I do highlight all the important, or most relevant, studies in the text (both in the introduction section, and in the discussions in the various papers).

17. Page 19, last paragraph: why is intramuscular saline (Smith) omitted from the list of pain induction procedures that have been used? Intramuscular injection of hypertonic saline has been added to the paragraph describing the various pain-induction procedures (page 20). Thank-you for identifying the non-intentional omission.

18. Page 22, line 5: the crucial Riley *et al* publication is not listed in the references. I hope this is the only omission. Ms. Iacovides needs to check. Thank-you for identifying the mistake. Riley *et al (1999)*, has been added to the list of references. I have checked that no other references have been omitted.

19. Page 22, last line of the first paragraph: "inconclusive" The spelling error has been corrected.

20. Page 22, first line of the second paragraph: I like this bold statement of confronting the methodological concerns. Thank-you.

21. Page 25, last five lines: those sentences were taken verbatim from the paper in the appendix. To avoid self-plagiarizing, either admit it is a verbatim quote, or re-write. The 2 above-mentioned sentences have been re-written (page 26).

22. Page 23, second paragraph, line 3: use the English term "adrenaline "not the US term "epinephrine". This has been corrected (page 28).

23. Page 37, line 6: heightened excitability of nociceptive neurons is peripheral, not central, sensitization. That is correct. Thank-you for pointing out the error. The statement has been modified to refer to central sensitisation, as intended (page 38).

24. Page39, second paragraph, line 3: "researchers". The spelling error has been corrected (page 40).

25. Page 40, last word: "viscera-visceral" The error has been corrected (page 41).

26. Page 43, second paragraph, line 4: "reflecting" The error has been corrected (page 44).

27. Page 58, first line: why are those data confined to adolescents? Most of the available data to date, on the percentage of dysmenorrhoeics who self-medicate, are on adolescents. I have added a more recent reference to support my statement in adolescents, but I have also added in a sentence with the statistics of medication use in university student populations

(page 59).

28. Page 62, new section: I think that Ms. Iacovides needs to introduce the fact that some studies have used diclofenac sodium and some diclofenac potassium here, and alert the reader to her intention to discuss the consequences in her conclusion. As recommended, I have added this information on page 66, before justifying my investigation in Chapter 4.

29. Page65: a very useful, and well-constructed, conceptual diagram. Thank-you.

30. Page 74, table 2: although this table got past the editor and reviewers, it contains numbers with totally-excessive significant figures. If the standard deviation of PGE, concentration in the control women is 217 pmol/1, it is unacceptable to give the mean concentration to one decimal point. At best, that concentration should have been specified as 580 ± 220 pmol/1. This is an unfortunate error; one which easily could have and should have been avoided. I will ensure the same does not happen in future reports.

31. Page 74, fig 1: it is intriguing that, considering the neurochemical association between pain and mood, and given that there is an underlying neurophysiological process leading to hypersensitivity to pain throughout the menstrual cycle, the neurophysiological process apparently does not underlie mood, which is disturbed only during menstruation. Indeed. It would appear that the mood disturbance is directly related to the experience of pain.

32. Page 85, last line below the table: specify the statistical tests. The name of the statistical test has been added below the table, as suggested, and removed from the heading of the last column (page 88).

33. Page 86, second paragraph, line 3: why was the arm selected randomly? The convention in pain studies is to use the non-dominant arm. When examining the literature (see Table 1, pages 12-17), I realized that yes indeed, many studies used the non-dominant side of the body to induce pain. However, several others do not specify which side of the body was used, some use both sides, and some even used the dominant side (e.g. Hellstrom & Lundberg, 2000; and Ribeiro-Dasilva *et al*, 2011). I therefore chose to randomly select an arm for my study.

34. Page 88, last line: what is the significance of specifying the "start of the experiment"? The women rated their menstrual pain on arrival to the laboratory, prior to the experimental procedures.

35. Page 89, table 2: I think that this table of P values is uninformative. What we need is the actual results of the Von Frey tests, and those results are not given. They should be.

I have changed the table, as suggested (page 92).

36. Page 91, second paragraph: it is very pleasing, in a PhD thesis, to be able to claim two "firsts" in one chapter. Thank-you.

37. Page 102, last line: I think that there was a design deficiency in this study. A paper in which one of Ms. Iacovides' supervisors was principal author (Baker *et al,* 1999a) showed that, if there was going to be abnormal physiology in a pain-free phase of the cycle, it would be in the luteal phase. We know that quality of life isn't affected in the benign late follicular phase. We still don't know whether it is affected in the luteal phase. The primary reason for selecting these two phases was to determine whether quality of life is affected by the presence of pain; by assessing quality of life twice in the same phase (early and late) we limited the possibility of having other factors, such a varying hormonal milieu, affecting our results. However, I thank-you for pointing out the worthiness of studying quality of life during the luteal phase too, and I will certainly consider doing so in future.

38. Page 103, line 1: see comment 3. More information on the validation of all the instruments used across my studies has been added into the Introduction (see Comment 3).

39. Page 105: see comment 32. The name of the statistical test has been added below the table, as suggested, and removed from the heading of the last column (page 108).

40. Page 106, last line: use of the Pearson correlation implies that there is reason to believe that the relationship might be linear. We are not shown this scatter diagram so can't judge for ourselves. Did Ms. Iacovides try a Spearman correlation? Pearson's correlations, are typically performed on these data in the literature (For example: Endicott J, Rajagopalan K, Minkwitz M, Macfadden W. A randomized, double-blind, placebo-controlled study of quetiapine in the treatment of bipolar I and II depression: improvements in quality of life. International clinical psychopharmacology 22:29-37, 2007, and Mick E, Faraone SV, Spencer T, Zhang HF, Biederman J. Assessing the validity of the Quality of Life Enjoyment and Satisfaction Questionnaire Short Form in adults with ADHD. J Attent Dis 11:504-9, 2008).

41. Page 109, paragraph 2, last sentence: **I** do not believe that the conclusion that there is reduced quality of life "only when pain is present" can be drawn from the study. See comment 37. Although this cannot be changed in the paper, I have added a sentence in my Conclusion (page 164) to clarify that in order to confirm my findings, future studies need to assess quality of life in the non-painful luteal phase too.

42. Page 120, paragraph 2, last two sentences: one of Ms. Iacovides' supervisors tested meloxicam, a COX-2 inhibitor that has not been withdrawn. Indeed, however, although superior to placebo, meloxicam was not as effective in reducing dysmenorrhoeic pain as were diclofenac and rofecoxib.

43. Page 121, first paragraph: Ms. Iacovides refers to the only other study which is comparable to hers. It is a pity that she did not compare the efficacies of the two regimens used. She easily could have done one comparison. In her analysis (pages 131 & 132), Ms.

lacovides did not test whether the pain after administration of diclofenac potassium was significantly different to zero. She could have done so. In women using the regimen of Chantler *et al* (2008), it was not different; diclofenac potassium abolished the pain. It is a pity that this was not done for the paper which is now published. For future experiments on the efficacy of a drug on pain relief, I will certainly run such an analysis. Thank-you.

44. Page 133, paragraph 1: I really am surprised that this paragraph passed the reviewers of the paper. The area under the VAS-time curve allows one to compare the magnitude of responses with different time courses, but conveys absolutely no information about the continuity of the response. Responses that last 24h, or are over in 1h, or occur only between midnight and 3am, can have the same area under the curve. Continuity was demonstrated by the ANOVA. It is unfortunate that this unintentional error was not identified and corrected prior to publication. I will be sure to avoid making such a mistake in future. Thank-you.

45. Page 133, paragraph 2: one cannot conclude that the curve of Figure 3 is a pure placebo response curve, because there was no "natural history" curve presented. We do not know what would have happened to the VAS over the 24h if they had been no intervention. The word “pure” has been omitted from the text in the thesis. However, as you correctly pointed out earlier, it is unfortunate that cannot change the paper at this point.

46. Page 139, line 2: the Willkens reference is inappropriate as evidence of the safety and tolerability of diclofenac, because it is so old. There has been a wealth of research on the cardiovascular safety of NSAIDs since 1985. Although the paper, which is already in press, cannot be changed, I have added in a more recent reference to my thesis supporting the safety and tolerability of diclofenac (Reference number 61, page 148: Gan TJ. (2010) Diclofenac: an update on its mechanism of action and safety profile. Curr Med Res Opin 26: 1715-1731).

47. Page 151: I am surprised how small the effects of dysmenorrhoeic pain on sleep were. For example, sleep efficiency was 95% even when the women were in pain. That means the benefits of treatment with diclofenac potassium, though statistically significant, were small. Although average sleep efficiency was only reduced from 97% to 95% when the dysmenorrhoeic women were in pain, the effect size was large, indicating that this difference was consistent even in this small sample. While 95% is still an excellent sleep efficiency, sleep was poorer on the night of pain. It is possible that a study of women across a wider age range might reveal a greater impact of dysmenorrhoeic pain on sleep. It also needs to be taken into account that 6 of the 10 women took rescue medication during the placebo arm of the pain trial, which may have lessened the impact of pain on sleep.

48. Page 163, paragraph 1: in the context of the emotional-affective components of pain, I think that a short discussion is warranted about the potential differences in emotional-affective component between clinical pain (for example the pain of dysmenorrhoea) and experimental pain (for example pain elicited by intramuscular hypertonic saline). Although experimental pain may cause some anxiety, its emotional component will not be the same as that of clinical pain. I do not think that the difference confounds any of Ms. lacovides' results. Thank you for this excellent and thoughtful suggestion. I have added in a short discussion (page 166), as recommended.

49. Page 164, last line: see comment 37. I have added a sentence to address the reviewers concern (page 169).

50. Page 168, second paragraph, line 6: "available" The spelling error has been corrected.

51. Page 172 reference list: I think that Ms. Iacovides has used an unnecessarily complex format for her references, for example using quotation marks for the titles of each paper. I am not asking for any changes, though, except that there must be consistency in her format. Look at the first four references. Two have the issue number of the journal and two do not; there must be consistency throughout the list. If journal titles are abbreviated, all must be abbreviated (see Dawood 1987, and the two Proctor references). All the references to book chapters need to be checked for consistency; they are not consistent currently. Although, not expected by the examiner, I have changed the format of my reference list to a simpler format, and I have carefully scrutinized the list to ensure consistency throughout.

Examiner 3

Examiners Report 4'h January 2013

The impact of primary dysmenorrhoea on pain perception, quality of life and sleep in young healthy women.

Stella Iacovides

0109478T

Introduction

I have been nominated to examine this thesis entitled 'The impact of primary dysmenorrhoea on pain perception, quality of life and sleep in young healthy women' by Stella Iacovides which has been submitted for consideration for the degree of Doctor of Philosophy to the Faculty of Medical Science, University of Witwatersrand, Johannesburg.

I am a Consultant in Pain Medicine and a practising clinician who regularly sees patients in pain and in particular adolescents and women with pelvic pain and dysmenorrhoea.

Brief description of thesis

For the preparation of this thesis, five separate studies were completed on three groups of health females with a history of severe primary dysmenorrhoea and age-matched controls.

This thesis clearly delineates its aims and highlights gaps in current knowledge that it attempts to close.

The aims were as follows:

1. To investigate the sensitivity to deep-muscle pain induced by hypertonic saline within and outside areas of referred menstrual pain in women with and without dysmenorrhoea; to investigate the effect of the menstrual cycle on pain perception in women with and without dysmenorrhoea; to investigate the sensitivity to ischaemic muscle pain in women with and without severe dysmenorrhoea during the menstrual cycle; to assess mood in women with and without primary dysmenorrhoea at different phases of the menstrual cycle.
2. To assess the quality of life in women with severe primary dysmenorrhoea and without dysmenorrhoea during menstruation and during a pain-free phase of menstrual cycle
3. To assess the recommended dose of diclofenac potassium compared to placebo in alleviating menstrual pain across a 24 hour time period in women with a history of severe primary dysmenorrhoea.

4. To assess whether objective and subjective measures of sleep quality are impaired by primary dysmenorrhoeic pain
5. To assess the effectiveness of diclofenac compared to placebo in alleviating night-time dysmenorrhoea and restoring sleep architecture and perceived sleep quality in women with severe primary dysmenorrhoea.

Analysis of the work performed

Chapter 2

The aim of this chapter was excellent. The researcher had investigated previous work, understood basic concepts, made an excellent proposition which was well researched and executed. The induction of deep muscle pain by injection of hypertonic saline was an innovative idea. Measurement of hormonal status was also good. The experiments were carefully performed, ruling out confounding factors such as the pain of the injection itself. Interesting results were obtained.

My criticisms of this research are that there is no mention of the race of the women studied and no work is cited which confirms that there is no racial difference in deep muscle pain perception with intramuscular injection of hypertonic saline, and that the numbers of subjects studied was small (12 with dysmenorrhoea and 9 without). There is no mention of a power calculation being done to give a guide as to how many women needed to be studied for the results to be significant. Is the number of subjects large enough for the conclusions to be robustly defended? Whilst this study's findings are in agreement with other studies, if this is the first time that an increased pain response to deep muscle pain has been shown, then it is a new and important finding and it is to be regretted that more subjects were not studied to make the findings more robust.

With regards to race: for the studies presented in Chapters 2a and 3, the group of women with dysmenorrhoea (n=12) consisted of 5 black, 1 mixed-race, 1 Indian and 5 white women, while the control group (n=9) consisted of 5 black and 4 white women. The dysmenorrhoeic group of women (n=11) study presented in Chapter 2b consisted of 4 black, 1 mixed race, 1 Indian and 5 white women, and the control group (n=9) consisted on 5 black and 4 white women. The group of dysmenorrhoeic women (n=24) that participated in the study in Chapter 4 consisted of 10 black, 2 mixed race, 3 Indian and 9 white women. Although I cannot add this information of the ancestry of the various groups of women into the already-published papers, I have added it into Chapter 2b (page 87).
Given the small sample size of all my studies, I believe it to be inappropriate to analyse the data according to ancestry. There is evidence that African-Americans report lower experimental pain tolerance and threshold, as well as more severe acute and chronic pain compared with white

Americans *(Edwards RR, Doleys DM, Fillingim RB, and Lowery D. Ethnic differences in pain tolerance: Clinical implications in a chronic pain population. Psychosom Med 63:316–323, 2001; Chapman WP and Jones CM. Variations in cutaneous and visceral pain sensitivity in normal subjects. J Clin Invest, 23:81–91, 1944; Chibnall JT, Tait RC, Andresen EM, and Hadler NM. Race and socioeconomic differences in post-settlement outcomes for African American and Caucasian Workers' Compensation claimants with low back injuries. Pain, 114: 462–472, 2005; Forsythe LP, Thorn B, Day M, and Shelby G. Race and sex differences in primary appraisals, catastrophizing, and experimental pain outcomes. J Pain 12: 563-72, 2011)*. Others, however, report no differences in the experience of pain across various ethnic groups *(Edwards RR, Moric M, Husfeldt B, Buvanendran A, and Ivankovich O. Ethnic similarities and differences in the chronic pain experience: A comparison of African American, Hispanic, and white patients. Pain Med, 6:88–98, 2005)*. Further, it is believed that ethnicity shapes one's attitude towards pain and how one communicates pain, including ones willingness to report pain (*Wandner LD, Scipio CD, Hirsh AT, Torres CA and Robinson ME. The perception of pain in others: how gender, race, and age influence pain expectations. J Pain 13: 220-227, 2012*), and pain-reducing behaviours (*Hastie BA, Riley JL and Fillingim RB. Ethnic differences and responses to pain in healthy young adults. Pain Medicine 6.1: 61-71, 2005*). A large national health survey conducted in white and African American adolescents found that although the prevalence of dysmenorrhoea was not different in the two ethnic groups, the rate of school absenteeism was double in the African American compared to the white adolescent girls (*Klein JR, Litt IF. Epidemiology of adolescent dysmenorrhea. Pediatrics 68: 661-664, 1981*). Ethnic and cultural differences in pain perception and experience have not yet been investigated in the South African population, and unfortunately I did not have a sufficient sample size to determine whether differences in pain exist between the different ethnic groups in my South African cohort.
However, given that my pain experiments were investigating differences in pain between dysmenorrhoeic and non-dysmenorrrhoeic women, and that there is an approximate equal breakdown of black and white women in the experimental (dysmenorrhoeic) and control groups, my results are not likely to been affected by differences in pain perception according to ethnic group, should such differences exist.

Despite the different ancestries of the women who participated in my study, I believe that my cohort are a homogenous group in other respects given that they are all from urban Johannesburg, who all attended English-speaking schools, were all attending university, and of a similar age at the time of my study.

I did not perform power analyses prior to my studies, however, regardless of the small sample size, I was able to detect statistically significant effects, in this particular case with regards to pain perception between dysmenorrhoeic and control women. It is also important to note that my study's sample size is comparable with the sample size of other experimental studies performed on cohorts of dysmenorrhoeic women.

I think it is debatable whether the term 'central sensitisation' should be used especially given the recent controversy about this fuelled by Professor Fernando Cervera. However, that is just my opinion and no doubt that particular debate will continue for some time.

This work is innovative and provides important knowledge relating to experimental muscle pain in women with and without dysmenorrhoea across the menstrual cycle.

Chapter 2b

This again is very innovative research. The research was carefully performed and shows very interesting results. As the author states, this is the first study to show that women with primary dysmenorrhoea are more sensitive to experimental muscle ischaemia compared to a non-dymenorrhoeic control group. I would reiterate my comments about the small sample size. A larger group would certainly enable more robust conclusions to be drawn.

Chapter 3

This study examines quality of life using the Quality of Life Enjoyment and Satisfaction Questionnaire in women with primary dysmenorrhoea both during menstruation and during the pain-free follicular phase of the menstrual cycle.

There is a good introduction. The same women are used as for the previous studies, whilst I do understand that women may be difficult to recruit, this does make the sample size very small for meaningful quality of life studies. I did not understand exactly why this particular questionnaire was used for this study. There is only one references to its use in pain dated from 1993- though a 2000 reference for its use on premenstrual dysphoric disorder. What advantages does it have in this population and why was this used rather than other QoL measures.

The Q-LES-Q is a non-disorder-specific QoL scale, which is comprehensive and assesses many domains of QoL. Given that the paper is in the form of a short communications paper, the number of references allowed was limited, and thus some of the research done with this tool on pain patients was omitted. The Q-LES-Q has been used in patients with pain in the past *(for example: Husain MM, Rush AJ, Trivedi MH et al., Pain in depression: STAR *D study findings. J of Psychosom Res 63: 113-122, 2007, and Matusiak L, Bieniek A and Szepietowski JC. Psychophysical aspects of hidradenitis suppurativa. Acta Derm Venereol, 90: 264-268, 2010)*, but indeed its use in pain patients is limited. The Q-LES-Q was primarily chosen for this study as it is comprehensive, easy to use and, as correctly pointed out by the examiner above, it has previously been used in women with a disorder related to the menstrual cycle (premenstrual dysphoric disorder).

With regard to sample size, the author states that that the sample size was adequate to detect a reduced QOL in women with primary dysmenorrhoea. This suggests that a power calculation was done to determine sample size. If so, this should be stated. My concern is that the sample studied appears to be a highly selected small group and a larger more socially, financially, educationally diverse group of subjects showing similar results would be more meaningful. No power analyses were done prior to the study. However, there was a strong statistically significant effect even in this small, relatively homogenous sample. You are correct in saying that my results are specific to my carefully selected group of women- it is not necessarily a reflection of the general population, but rather that of women who are young, generally healthy, with regular ovulatory menstrual cycles, normal psychological status, no severe PMS, no indications of secondary causes for dysmenorrhea, and no chronic illness. I hope that my research findings may stimulate further research about quality of life in larger populations of women, considering such factors such as age, ethnicity and socioeconomic status.

Chapter 4

This study investigates menstrual pain in women with primary dysmenorrhoea treated with diclofenac potassium. My understanding is that diclofenac potassium is more soluble that diclofenac sodium and is therefore more quickly absorbed.

There is a very comprehensive introduction that details different types of NSAIDS that are currently available and the safety concerns related to the COX-2 specific inhibitors.

However, this concern has now also grown to include diclofenac, as it has been suggested that diclofenac use, at both at low and high doses, increases the risk of acute myocardial infarction when used long-term. In my hospital and in the Primary Care General Practices in my city, diclofenac use is now actively discouraged, except for use by suppository peri-operatively.

There has been a recent recommendation that diclofenac should be removed from the Essential Medicines Lists in low, middle and high income countries. The risks associated with long-term diclofenac use have important public health implications and although young women with primary dysmenorrhoea are unlikely to develop cardiovascular disease with diclofenac, perhaps this risk should be acknowledged in this thesis and suggestions made for the widely available safer alternatives.

In this thesis, the gastric side-effects are comprehensively discussed but there is no mention of cardiovascular side-effects. The Cochrane Review stated that no one NSAID was obviously more effective than another in treating dysmenorrhoea and perhaps the *very* positive endorsement of diclofenac in this thesis should be moderated. Neither the Cochrane Review publications nor this study was powered to pick up adverse effects.

I acknowledge the examiners concern of diclofenac and its association with cardiovascular disease with chronic/long-term use. However, I do not believe that my endorsement of diclofenac for dysmenorrhoea used for 1-2 days per month classifies as chronic use. Also, the examiners concern is completely valid for elderly patients, particularly those with heart failure (For example: *Cannon CP et al. Cardiovascular outcomes with etoricoxib and diclofenac in patients with osteoarthritis and rheumatoid arthritis in the Multinational Etoricoxib and Diclofenac Arthritis Long-term (MEDAL) programme: a randomised comparison. Lancet 368: 1771-81, 2006; Warner TD and Mitchell JA. COX-2 selectivity alone does not define the cardiovascular risks associated with non-steroidal anti-inflammatory drugs. Lancet 317: 270-3, 2008;Gislason GH et al. Increased mortality and cardiovascular morbidity associated with use of nonsteroidal anti-inflammatory drugs in chronic heart failure. Arch Intern Med 169:141-149, 2009).* I do not know of any risks associated with short-term use of diclofenac in young women.

With regard to Methodology, I was not sure that I understood the rationale for taking diclofenac when the women did not have pain. Whilst this is what we suggest in practice to women with severe pain, it was not clear what this aimed to achieve. I was also surprised that there was the potential for a women to take diclofenac 200mg daily, which is greater than the normally recommended daily dose, plus the potential to take additional NSAIDs with diclofenac- though no women actually did.

Some believe that there is merit for treating pain before it begins, or preemptive analgesia (Jerosch J, Saad M, Greig M, & Filler T. Suprascapular nerve block as a method of preemptive pain control in shoulder surgery. *Knee Surgery, Sports Traumatology, Arthroscopy*, *16*:602-607, 2008; Kaye AD, Baluch A, Kaye AJ, Ralf G, & Lubarsky D. Pharmacology of cyclooxygenase-2 inhibitors and preemptive analgesia in acute pain management. *Current Opinion in Anesthesiology*, *21*: 439-445, 2008). In fact, research recommendes that women with dysmenorrhoea initiate treatment 1-2 days before the onset of menstruation (Harel, 2004). However, our protocol was mainly designed to ensure that NSAID administration was similar between women, and that the daily recommended dose was taken over the 24 hours of investigation.

Regarding the potential for women to exceed the daily recommended dose of 150 mg, the examiner is correct. This was a fault in the study design that was regrettably overlooked by myself, my supervisors and the ethics committee. Given that the ethics committee insists that women need to have the freedom to take rescue medications if need be, it should have been stipulated that the women were not allowed to take NSAIDs, in particular, as rescue medications for pain relief. I will ensure that the same error is not made during future investigations of a similar nature. As it happened, fortunately, no woman took an additional rescue medications, nor did they take the 4th diclofenac pill, during the diclofenac arm of the trial, and thus the daily recommended allowance was not exceeded.

I was interested that the regular administration of diclofenac proved positive in this study and that pre-menstrual administration may not be necessary

Chapter 5

This paper relates the effect of diclofenac on sleep quality in women with dysmenorrhoea. The study was well undertaken and conclusions appear valid.

An appraisal of the thesis

The Conclusion of this thesis was well written. I would agree that the author's research has provided useful additional knowledge about women with dysmenorrhoea and also in the use of NSAIDs to treat dysmenorrhoea. Her thoughts were well ordered and her work well organised. She has a clear writing style.

In particular, her work on hyperalgesia with deep intramuscular hypertonic saline in women with dysmenorrhoea and on increased ischaemic muscle pain was well researched and accomplished in a satisfactory manner. I found this most interesting and useful. It will surely lead to more research. It is disappointing that especially in her original work on muscle hyperalgesia and on ischaemic pain, both of which I am not aware has been studied before, that greater numbers of subjects were not included to produce more robust results. I would agree with her that her results are compelling and do take into account many of the methodological problems of previous researchers. This result does indicate further research is needed.

I do have some concerns regarding the small number of subjects studied generally, which she acknowledged several times. This was most apparent in her chapter on mood and quality of life. I am not sure if it is true that this thesis 'extends epidemiological research' due to the small sample size. Please see comments above.

With regard to her work on diclofenac, I do feel that she needs to provide some additional work to discuss the ongoing concerns about diclofenac, to state that the Cochrane Collaboration found not difference between different NSAIDs with regard to effectiveness and to suggest some alternatives. The thesis read as though diclofenac is the drug of choice. I think if this is done, then the thesis will read as more contemporary, indicating that the author is up to date with new, current medication issues and it will mean that anyone reading the thesis, will not be left wondering if diclofenac is still the optimal NSAID to use. Please see comment and references above.

Otherwise, this is a comprehensive body on additional knowledge on a common condition that adds to the literature.