INVESTIGATING POSTPARTUM DEPRESSION IN SOUTHERN RURAL

EGYPT AND EFFECTS OF SERTRALINE ON *FSH* AND *LH* GENE

EXPRESSION ON FATHEAD MINNOWS USING RT-PCR

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Thesis Prepared for the Degree of

MASTER OF SCIENCE

UNIVERSITY OF NORTH TEXAS

May 2013

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Mohamed, Hagar Abdo. <u>Investigating postpartum depression in southern rural Egypt and effects of sertraline on *FSH* and *LH* gene expression on fathead minnows using RT-PCR</u>. Master of Science (Interdisciplinary - Women's Studies), May 2013, 47 pp., 1 table, 6 illustrations, bibliography, 42titles.

Postpartum depression (PPD) is a major health problem that affects many women worldwide. In Egypt, PPD is neglected despite the expected high prevalence rate among women during the transition period after the Egyptian revolution. This research investigated the prevalence, risk factors, and interventions of postpartum depression in southern rural Egypt. Interviews were conducted with 57 participants recruited from public and private hospitals. Questionnaires and the Arabic version of the Edinburgh Postnatal Depression Scale were administered. The prevalence of PPD is 73.7%. PPD is associated with low income and age at childbirth. Most participants regarded screening mothers after childbirth for PPD as effective; in comparison to, antidepressants that were regarded by most participants as ineffective. Women in southern rural Egypt prefer high number of pregnancies, so investigating the influence of sertraline, an antidepressant medication, on female hormones becomes important. In this research, fathead minnows were exposed to 3 and 10 ppb sertraline for 7 days. Real-time polymerase chain reaction was used to detect the change in gene expression of the follicle-stimulating hormone (*FSH*) and luteinizing hormone (*LH*). Results showed that a down regulation at the 10 ppb was evident on the LH and to a lesser extent on FSH. Our results increased levels of sertraline inhibited GnRH which influenced expression of *LH* and *FSH*.

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CHAPTER 1

INTRODUCTION

1.1. Definition of Postpartum Depression

The American Psychological Association defines postpartum depression (PPD) as "a serious mental health problem characterized by a prolonged period of emotional disturbance, occurring at a time of major life change and increased responsibilities in the care of a newborn infant. Postpartum depression can have significant consequences for both the new mother and the family" (APA) [2]. Postpartum depression usually appears in the first 6 month of delivery. The symptoms range from tearfulness, feelings of guilt, feeling of inadequacy, loss of appetite, failure to cope with the baby, irritability, sleep disturbances, inability to concentrate, perceiving oneself as an unloving mother, and excessive fatigue. Suicidal thoughts might evolve [37].

1.2. Significance of Postpartum Depression

Postpartum depression is considered a major public health concern [15]. Also, it can negatively affect the feelings, behavior, and cognitive behavior of the mother. PPD can lead to interpersonal problems for the mother [36]. Interpersonal problems are defined as the persistence of occurrence of complications when dealing with others [28]. PPD is disadvantageous to the social life of the mother because the baby and the marital relationship can be dramatically affected by PPD. For instance, PPD influences the behavior and the feelings of the mother towards her child [36]. For example, a study found that PPD is correlated with negative breastfeeding attitudes and delayed infant growth [3]. If left untreated or unrecognized, PPD can cause lasting adverse outcomes [36].

It is important to consider the welfare of the social life of the mother. During the late 80s and 90s, the feminist psychologists in the Stone Center at Wellesley College created the self-in-relation theory or gender-relations theory. This theory explains that the self-concept in women develops based on her relations with others and her tendency to sacrifice her own achievements for the favor of others (i.e. family oriented). In the lives of women,

relationships give them strength and self-esteem. As a result, women encourage building and maintaining relationships as an integral part of their development and identity [**24**]. Therefore, it is important that the society and the medical health community detect PPD and early treat PPD for the favor of the personal and social wellbeing of women, and to make sure that women are not struggling between their nature of offering their best behavior to their families while suffering from PPD.

1.3. Prevalence of Postpartum Depression

PPD is the most prevalent complication of childbirth [**23**] [**34**]. According to Robertson et al. [**36**], PPD occurs in 10 to 15 % of the new mothers within 6 months of delivery. However, higher incidences were seen in India (32%), Korea (36%), Guyana, and Taiwan (61%) [**5**]. On the other hand, Halbreich and Karkun found that prevalence of PPD in some countries is close to 0%, such as Singapore, Denmark, Malta, Malaysia, and Austria [**19**]. In the Middle East, 18% was the incidence rate of PPD in Dubai, United Arab Emirates [**4**]. In Abu Dhabi, United Arab Emirates, the incidence of PPD was 22% at 3 months-postpartum. This rate fell to 12.5% when the subjects screened at 6 months postpartum [**18**]. Thus, it can be concluded that PPD occurs at a wide range internationally.

CHAPTER 2

LITERATURE REVIEW

2.1. Conflicting Information in the Literature

Defining the nature of postpartum depression across cultures can be challenging. In fact, the literature contains contradictory opinions about the risk factors and the causes for postpartum depression [36]. For example, Abou-Saleh and Ghubash concluded that the risk factors of PPD in sample of women from Dubai were comparable to those of women from industrialized countries [4]. Considering the different culture, religion, and social settings of both groups, it is unexpected that both groups could have similar risk factors associated with PPD. On the other hand, PPD can take different forms across personalities [16]. Careful study of PPD in communities allow the decision makers to build a customized definition, etiology, and hence, preventative measures for PPD .Therefore, the importance of investigating PPD across cultures [4] and even across provinces in a given country [38] is essential to best serve the psychological health of women. This may be challenging given the lack of information in many areas. The following subsection explores some of the key risk factors identified in the Arab and Muslim countries.

2.2. Key Risk Factors of Postpartum Depression Identified in Some Arab Countries

Abou-Saleh and Ghubash were the first to study postpartum depression in the Arab countries [4]. Their study was conducted in Dubai, United Arab Emirates . The study included 95 participants who were interviewed on the 7th day after delivery. The incidence of PPD was 18% and the risk factors identified as mostly correlated with PPD were previous psychological problems, marital problems, other stressful life events, concerns about the pregnancy and the newborn child, and signs of distress early in the puerperium.

A newer study was conducted in Sharjah, United Arab Emirates [20]. It screened 173 participants using the Arabic version of the Edinburgh Postnatal Depression Scale (EPDS). The study indicated that 10% of the participants were diagnosed with PPD with the most

significant risk factors being depression during pregnancy in the second and third trimesters, number of children, and use of formula for feeding [20].

In Tunisia, Masmoudi et al. found a rate of 19.2%, and 13.2% of PPD rates in the initial, and follow up visits, respectively in a sample of Tunisian women screened by EPDS [30]. On the other hand, in Lebanon, PPD rate was 21 % with rural Lebanese women were found to have a greater PPD incidence than other Lebanese women [12]. Eloul at el. [16] indicated that high birthrate and early age of first conception are common risk factors for PPD in the Middle East. Additionally, they argued that the postpartum rituals that are traditionally practiced in the Middle East could be protective factors against PPD.

An extensive study including 679 subjects was conducted in Turkey. The PPD prevalence was 25.6% in the sample. The risk factors identified were previous psychiatric illness, smoking, low economic status, relationship problems with husband or mother-in-law, dissatisfaction in social relations, previous loss of a baby, and giving birth to a baby girl.

A recent study was conducted in Egypt by Saleh et al. included 60 PPD cases and 60 controls [38]. The study was leading in its objectives and conclusions. The PPD rate was found to be 17.9%. The study demonstrated that PPD is strongly associated with psychosocial, socioeconomic, obstetric, and hormonal factors (i.e., decrease levels of thyroxinT3, cortisol, and estradiol level).

2.3. Statement of the Problem

The literature serves as important source for understanding the incidences and risk/protective factors of PPD in few Arabic and/or Muslim communities. Moreover, the literature highlights the social context, the mental health care levels, and some of the challenges that could be faced on conducting such studies in Arabic and/or Muslim communities.

Nevertheless, there is still lack of research on PPD on the Arab countries [20] and generalizations from previous studies would be inconsistent. Further, only two studies about PPD were conducted in Egypt. The first is by Abou Nazel and Nosseir [3] included only 158 participants. The second is by Saleh et al. [38]. But, their data were acquired from women interviewed during late 2006 and early 2007. In my view, after the major economic

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and political instability experienced by the Egyptian society, it is essential to measure the incidence of PPD after the revolution, especially in the most underprivileged population, rather than acquiring patients data retrospectively. Previous studies failed to provide specific interventions to effectively deal with the problem of PPD. This reveals the need for more extensive research on this area.

2.4. Hypothesis and Objectives

The southern part of Egypt (Upper Egypt) is known to be less advanced with several development indicators are far less than other cities in Egypt [29] . Illiteracy, early marriages, unemployment, and poverty are some the major problems in upper rural Egypt. Personal communication with several local obstetricians and gynecologists indicated that there is absence of any form of postpartum depression care or awareness in the medical community or in the society, despite the fact that postpartum depression is taught to medical school students. My research hypothesized that postpartum depression is occurring at a higher rate in upper rural Egypt. In fact, Saleh et al. partially emphasized my hypothesis. They indicated that rural women represented most of the PPD patients in their sample. Saleh et al. studied rural women living in the northern part of Egypt that has better services and infrastructure; however, I am proposing to investigate PPD in rural women living in Upper Egypt.

The objectives of this study are (1) to investigate the incidence rate of PPD in Egyptian women using the Arabic version of EPDS; (2) to identify the most important risk factors associated with PPD in upper rural Egypt; and (3) to score the effectiveness of several culturally-sensitive proposed interventions to deal with postpartum depression from the participants point of view.

CHAPTER 3

METHODOLOGY

3.1. Location and Time Frame

Fifty-seven participants were recruited from (a) Kom-ombo General Hospital located in Kom-ombo City, Aswan Governorate, Egypt; (b) Maternal and Child Care Clinic; and (c) private clinics. The study was conducted from November 2012 to January 2013.

3.2. Procedure

The study was conducted according to the Institutional Review Board of the University of North Texas. Women who are two weeks to one year postpartum were included in the study. An exclusion criterion is comprised from (a) women who are less than two weeks postpartum or more than one year postpartum or (b) women who are within the two weeks postpartum to one year postpartum but had one of the following cases: abortion, stillbirth, or new pregnancy. The purpose of the interview was explained to the participants prior to completion of the interview. Informed consent was provided from the participants and their guardians if needed.

3.3. Tools

An interview was held and a questionnaire was administered to the participants. The questionnaire contained 4 sections (1) demographic data section. It contained questions about age, income, education, number of children, age of the baby, and infrastructure condition of the residence (2) the personal feelings section. It contained questions about self-perception of body image, the degree of romance in the relationship with the husband, degree of the help received from the husband in the household duties, religiousness. (3) the society and environment section. It included questions about degree of help received from the others towards the household duties or the child, the influence of the Egyptian revolution, whether the participant or her relatives or her neighbors been robbed lately, and influence of the Sugar Factory. The Sugar Factory is a factory disposing black dusty powder

in the sky of the town where the study was conducted. Several efforts were done to reduce its environmental pollution, but until now it, none of it succeeded (4) the interventions section. In this section, women were asked to rank from their point of view the effectiveness of various suggested methods to prevent and deal with postpartum depression. The interventions are the effectiveness of administering the Edinburgh Postnatal Depression Scale EPDS after delivery by the physicians, the educational role of obstetricians about postpartum depression for the husband and the family, awareness campaigns in media about postpartum depression, awareness campaigns in the community by the religious leaders and/or physicians, and joining support group. Some women were asked about their willingness of taking antidepressant medications and their knowledge about the medications possible adverse effects. Women scored the interventions as effective, slightly effective, ineffective, or do not know.

The Arabic version of the Edinburgh Postnatal Depression Scale EPDS, validated by Ghubash et al. [4]was used. It is a user-friendly self-report consisting of 10 questions with four possible answers, each answer is rated on a 0-3 point scale. Zero and 30 are the possible lowest and highest scores respectively. A cut-off score of 9 indicating postpartum depression and a cut-off score of 12 for major depression (i.e., clinical depression) [22].

3.4. Essential Modification in the Tools

After consultation with Dr. Abdo El Swesy, gynecologist and obstetrician, and Director of Management Sciences for Health office in Egypt, the Arabic version of EPDS was modified to be suitable for the Egyptian dialect. It would have been problematic to administer the questions in the Jordanian dialect that is used in the Ghubash et al. [4] version. The modifications made the questions closer to the informal language that Egyptians use and hence easy to comprehend and respond to. In addition, assistance in understanding the questions was needed not only because of the high illiteracy rate among the participants, but also because of the unfamiliarity of the participants to being interviewed and to the purposes of conducting a research.

Due to the personal nature of the questions contained in the questionnaire and in the EPDS, the only way of recruiting and interviewing participants was face-to-face con-

versations. As I am a female and a licensed pharmacist, the participants trusted that their identity would not be revealed and their input would be used for scientific and academic purposes only.

3.5. Statistical Analysis Methods

All statistical analysis was conducted using IBM® SPSS ®Statistics Version 20. Significance of risk factors was determined using Pearson correlation coefficient. A p value of 0.05 or less was considered statistically significant.

CHAPTER 4

RESULTS

4.1. Demographic Characteristics

4.1.1. Education

Approximately 70 women were approached, 57 of whom become participants in the study. The educational levels of the participants were as follows:

- 9 % illiterate
- 18.2% elementary education
- 3 0.3% middle school
- 27.3% high school
- 15.2% higher education

The educational data represent only 33 participants (57%). Only data about 29 husbands were collected (50.9%). The educational levels of the husbands were as follows:

- 10.3 % illiterate
- 0% elementary school
- 3.4 % middle school
- 38 % high school
- 48.3% higher education

4.1.2. Income

Approximately 64% of the participants earned less than 1,000 Egyptian pound (L.E) (less than 162 USD) per month. In other words, according to the participants, their income is decided on a daily basis. The income of 14.5% of the participants was between 1,000 and 1,500 L.E (162 to 243 USD) per month. Around 11% earn 1500 to 2000 L.E (243 to 324 USD) per month. The affluent group represents 10.9% whose income exceeds 4,000 L.E (648USD) per month [11].

4.1.3. Age and Number of Children

The mean age of the participants is 25 (STD 4.7%), with minimum 17 and maximum 38 years-old. The demographics of 48 participants (84%) were as follows:

- 29.2% have 1 child
- 33.3% have 2 children
- 27.1% have 3 children
- 6.3% have 4 children
- 2.1% have 5 children
- 2.1% have 6 children

4.1.4. Infrastructure and Environmental Problems

The responses of 43 (75%) women asked about the condition of their infrastructure revealed that 53.5% of the participants endure at least one environmental or infrastructural difficulty. The frequency of environmental problems were as follows:

- sewage problems 10.2 %
- sugar factory 15.3 %
- sugar factory and sewage 8.5%
- sugar factory and electricity 3.4%
- electricity 1.7 %
- no environmental problems 33.9 %

4.1.5. Political Situation and Security

This study was conducted 24 months after the Egyptian revolution. In this study women were asked about how they perceived the effects of the transition period and the instability after the Egyptian revolution on them and their families. Forty-four women were asked about the influence of the revolution on their lives and the answers were as follows:

- 20.5% of the participants had negative consequences on the income
- 18.2% reported that their security was seriously affected after the revolution
- 9.1% were of the participants were affected in the income and security

- 52.3% of the women believed that they are not affected by the revolution

- 18.2% of the participants or their friends were robbed after the revolution

A statistical significant correlation between participants affected by the revolution and having infrastructural problem (p value 0.045) and being robbed (p value 0.05). This emphasizes the fact that the poor (having infrastructural problems) are the most affected by the revolution (economy and security) and at the same time most vulnerable (most being robbed).

4.2. Edinburgh Postnatal Depression Scale (EPDS) Scores

The prevalence of postpartum depression using the recommended cut-off score of 10 was found to be 73.7%. Mean EPDS score is 13.9 (STD 4.98%) with minimum score 3 and maximum score 26.

4.3. Risk Factors Associated with Postpartum Depression

Several risk factors were examined in their association PPD. Table 4.1 illustrates the risk factor and their p value.

TABLE 4.1. Risk factors and the corresponding p-values

Risk factor	p-value
Education	0.36
Husband education	0.11
Child age in months	0.1
Formula-usage	0.34
Attractiveness	0.1
Husband help	0.43
Religiousness	0.24
Number of children	0.43
Help from others	0.09
Infrastructure problems	0.36
Influence of revolution	0.17
Being robbed	0.15

4.3.1. Income

A significant correlation between EPDS scores and income (p value 0.05), and age of the participant ($p < 0.03$) were found.

4.3.2. Feeding Methods

Approximately 61% of the participants reported exclusive breastfeeding. In fact, 38.9% of the participants reported introducing children formula and 8.8% complained from breastfeeding practices and scarcity of milk. The child feeding pattern is further discussed in the discussion section. Although 42.1% of the 57 participants stated that child sickness was the main reason for their depression, no significant correlation was found between child sickness and depression in this study.

4.3.3. Emotions and Marital Life

No significant relationship between EPDS scores and body image satisfaction (p value 0.1), husband help in the household duties ($p < 0.43$), or religiousness ($p < 0.2$). Surprisingly, an unexpected significant positive relationship between romance in the relationship and EPDS scores was found (p value 0.001). Additionally, a significant positive correlation between body image satisfaction and husband help in household duties was shown (p value 0.003). A strong association was found between romance in the relationship and husband helping in household duties (p value 0.0001). This special situation is further explained in the discussion section.

4.4. Interventions

Figure 4.1 shows the preference of the participants of various interventions suggested to detect and deal with postpartum depression. There is a significant association between being depressed and choosing support groups as an effective intervention (p value 0.038). In the general population (depressed and non-depressed), most participants viewed screening women using Edinburgh Postnatal Depression Scale (EPDS) after childbirth as an effective method to detect and deal with postpartum depression. Most participants indicated that antidepressant medications are ineffective.
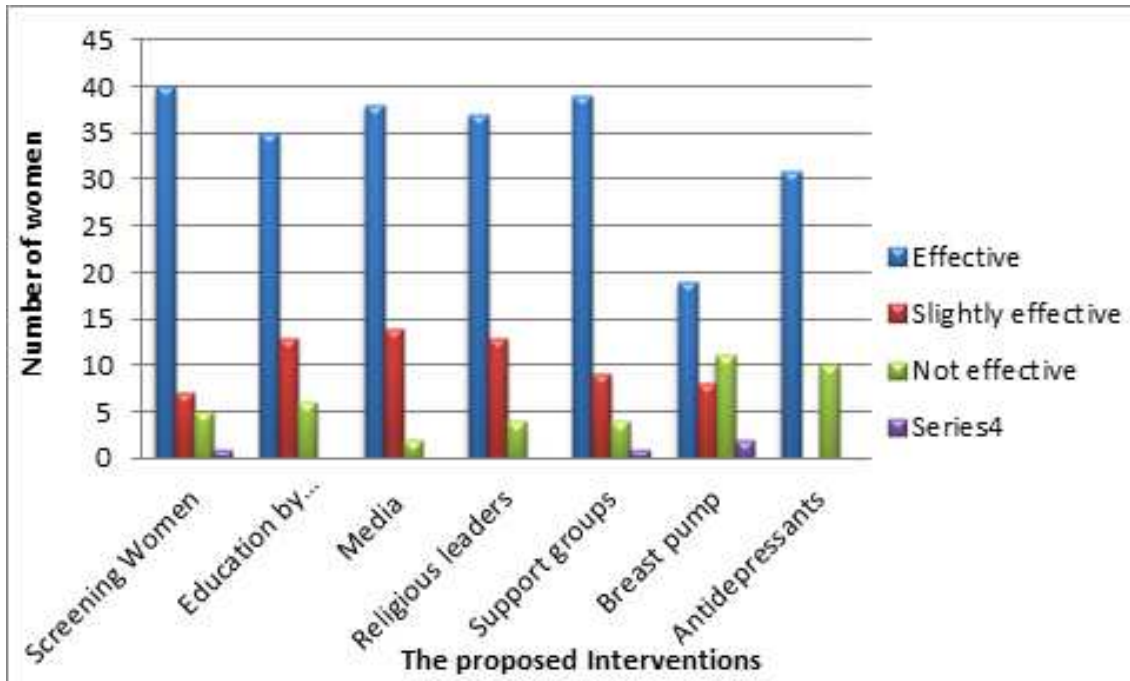
FIGURE 4.1. The Preference of the Participants of Suggested Interventions to Deal with Postpartum Depression

CHAPTER 5

DISCUSSION

5.1. Income

There is a statistically significant correlation between income and EPDS scores, as indicated by Saleh et al. [**38**]. In fact, in our study only 10.9% of the participants were affluent (income 648 USD/month) and 63.6% of the participants have income determined on a daily basis which is problematic taking into consideration basic daily life expenses. For example, the price of one kilogram of some food items shows food expenses as follows:

- Rice 4 L.E (0.6 USD)
- Meat 60 L.E (9.72 USD)
- Beans 7 L.E (1.1 USD)
- Government-supported flour 0.6 L.E (0.09 USD) [**11**].

This indicates that both the poor and the middle class face an economic difficulty today in Egypt.

5.2. Feeding Methods

Breastfeeding was favored by most of the participants. A small percentage of participants (38.9%) reported that using formula was due to scarcity of breast milk, children hating mothers breast, or having a twin. It appeared that it is a common knowledge among the participants that medications administered after surgeries (e.g., cesarean section or difficult delivery) result in a dramatic reduction in their breast milk. Therefore, they seek introducing other supplementary food. Financial ability is determinant in choosing the kind of food the child receives. Thanks to governmental supported programs, many women are able to get milk formula because all neonates are included in the national health insurance program that renders the formula highly affordable. Mothers can get milk formula after physician approval for a maximum of 5 months. Physicians approval is obtained after a medical examination where a female physician presses on the patients breasts and if no milk comes out, then the

mother will be approved to get the economic cans of formula. On another hand, several other participants find it easier and more accessible to purchase children powdered milk 1.5 L.E (0.22 USD)[11] or water diluted cows milk (possession of a cow makes this solution the best convenience). Introduction of the supplementary food begins as early as 2 months.

5.3. Age of the Participant

Our results contradict several studies indicating that age fails to predict postpartum depression, such as Ohara and Swain [34] and Hamdan and Tamim [20]. The strong significance of the positive correlation between age of the participant and EPDS scores demonstrated in this study is explained by the high number of children under the responsibility of the woman at older age. The older the woman, the higher the number of children she takes care of. This creates a major burden on the lactating mothers, especially among the financial and security situation of the country. It is important to highlight that the high pregnancy rate among southern rural women is regarded as a social advantage to them. This claim is further supported by the statistical significant correlation between age and number of children (p value 0.0001) found in this study. Moreover, this trend is supported by the fact 38.1% of women identified as depressed (26 out of 42) reported that they felt happier than before on answering the first question of the EPDS survey. This indicates that regardless of the responsibility that women after childbirth take, they feel happy as it is a social norm that good woman can get more children.

5.4. Emotions and Marital Life

The unexpected correlations revealed in the Emotions and Marital Life subsection is hard to explain. However, the on-field observations and discussions with husbands help understand this complex situation. A typical scenario of an interview with one of the couples is as follows: a young female married to a young male farmer. Their activities are limited to the farm, the children, and their sexual activity. It was noticed that there was an apparent harmony and active sexual activity in these scenarios. Moreover, the male partners from these scenarios were more involved in the household duties than the other more urbanized

male partners. However, in most of these scenarios, the income of the couples was decided on a daily basis. This emphasizes that a strong correlation will be found between husband help in the household duties and romance in the relationship (p value 0.0001). As a result, women feel contented with their relationship with their partners as evidenced by the strong correlation between body image satisfaction and husband help (p value 0.003).

Thus, other factors may explain the high depression scores in this group. For instance, although the help from the husbands or family may be reported as sufficient, it could mean that women get only the help of the husbands that are culturally-approved as adequate or suitable. In our view, rural husbands are more open to accept ideas involving supporting women in the physical labor than their more urbanized counterparts.

The strong positive correlation between romance in the relationship and high EPDS scores is problematic. It could be argued that romance, including sexual intercourse, is strongly associated with higher depression scores because of the possibility of the intercourse forming a burden on women after childbirth. However, the eagerness of the participants to talk about their sexual relationships, as they were viewed as a major source of joy in their lives, cast doubt on this claim.

5.5. Lack of Information

During several occasions in this study, lack of information from the participants was clear. For instance, perceiving that the Egyptian revolution did not affect the life of the woman, false belief that antibiotic medications hinder breast milk formation, inability of some participants that the Sugar Factory could represent any danger to their health, and most importantly, that many of the postpartum depression symptoms are considered as normal that every woman feels the same, even after one year of childbirth. This calls for the need for educational programs that reach every rural area in south Egypt, especially because women from smaller towns complained that their towns were excluded from educational and developmental programs.

5.6. Interventions

Asking women about their opinion about suggested solution for a problem related to their emotional health was innovative. The unfamiliarity of women to the notion of depression after childbirth and some of the interventions resulted in further discussions with the participants which revealed important information that should be considered by health decision makers in southern rural Egypt.

5.6.1. Screening Women for Postpartum Depression and the Role of Clinician in Educating the Patients

Screening women using Edinburgh Postnatal Depression Scale after childbirth was the most preferable method of detecting postpartum depression. Some participants indicated that being asked about emotional well-being by their obstetrician and gynecologist is strongly needed, given the trust and the respected role that physicians have among the society. However, other participants indicated that a female obstetrician and gynecologist would be preferred to the woman and her partner, and some other women indicated that a male physician would be more effective if the physician is going to encourage the family members to support the woman and help her during this critical time. In other words, a female physician is better for heart-to-heart conversation, but a male physical is better for an educational and directive role. This group ranked education by clinician as slightly effective. The group that selected education by clinician as an ineffective intervention was concerned about disclosing their emotions to their physicians, or because the idea of encouraging the partner to support the women in this period would be unwelcome by the husband.

5.6.2. Media and Awareness Campaigns

In our view, media plays an important role, where all society members receive the educational information needed without having to deal with privacy and male/female concerns. Holding awareness campaigns was one of the solutions that was appreciated by women. However, busy schedules and husband approval for them to go out were the hindrances for those women to attend such campaigns. This is the reason why awareness campaigns were

the most solution frequently regarded as slightly effective. Around seventy percent of the participants viewed awareness campaigns as effective.

5.6.3. Role of Religious Leaders

Most of the participants were Muslims. However, the role of Muslim as well as Christian religious leaders as educators for the community was examined. Participants were asked about the possibility that religious leaders talk about postpartum depression and educate the public about it. Approximately sixty eight percent of the participants indicated religious leaders are remarkably influential. Women indicated that if gynecologists and religious leaders started raising awareness about PPD it is going to be most effective. On the other hand, 24.1% of the participants reported that religious leaders are only slightly effective in educating the public. Some of those participants stated that religious leaders do not talk about such topics. Approximately seven percent of the participants declared that religious leaders are ineffective because postpartum depression is not the area of expertise of religious leaders. They are unsuitable to talk about emotional health and well-being. They confirmed that it is the area of expertise of the gynecologists and obstetricians.

5.6.4. Breast Pump

Women were asked whether breast pump can be a healthy and economic alternative to mothers complaining from sleep deprivation and scarcity of milk. The function of breast pump was explained to the participants, as they were unfamiliar with it. Their responses were as follows:

- 47.5% said it is an effective method, most of those people had limited breast milk or were working
- 20% reported that breast pump is slightly effective
- 27.5% reported it as ineffective. They considered formula milk safer than breast milk from the breast pump

5.7. Limitations

The time and financial obstacles were major reasons for the limited number of the sample. Moreover, security, transportation, and difficulty in finding a suitable governmental parity that could approve conducting the study in a larger geographical area played an influential role in determining the town where the study was conducted. Unfamiliarity of the participants with the topic of the study was a reason for the interviews to take longer time than expected. As a result, collecting demographic data as well as the depression screening test, were given the priority, then assessing the effectiveness of the suggested intervention, and risk factors questions were given the second priority.

5.8. Recommendations

Postpartum depression research in Egypt is still a new area of research. Therefore, extensive research is needed to discover more risk and protective factors associated with PPD in order to better guide health decision makers. Government should start intervening in postpartum depression issues perhaps primarily by screening women for PPD because that will lead to appreciation of the size of the problem and consequently application of nation-wide cost-effective and culturally-sensitive methods.

CHAPTER 6

CONCLUSION

Postpartum depression prevalence in southern rural Egypt is occurring at a danger-ously high rate; accompanied by dramatic lack of awareness about it. Postpartum depression is strongly associated with low income, and age at childbirth. Further, the participants of this study regarded screening women after childbirth with Edinburgh Postnatal Depression Scale, then joining support group as the most effective ways to detect and deal with post-partum depression. Moreover, antidepressant medications were considered the least effective method.

It is best to conclude with some quotes from the mothers:

"Although this is my fourth baby, when he got sick, I was behaving like he was my first child. I did not know whether I should cover him with the blanket or not, and what to feed him"

"I am always worried about my baby. I always check on him, even when he is asleep I wake him up, just to make sure that he is alive and fine"

"In order to get approval for a free baby formula, I have to undergo a medical ex-amination where the physician presses on my breasts to make sure that they do not excrete milk; before, I had no problem with that, but I was very humiliated when the doctor made the same examination on my breasts when I had an abscess on one of them"

"I already feel relieved just by chat a little with you "

"I would join support groups; I could chat with the person sitting beside me on a bus, as if she is an old friend"

"If they made breast pump available; I would 'run run' to get them "

"I wish that our government will take care of women " Many women said that.

"I wish that our government will take care of us (small rural towns), we just have primary school built in our town only last year; before that student had to travel "

"I wish to get more aware and educated about my health and nutrition"

"I came to visit the doctor because I do not know what is wrong with me. I do not have a physical complain. But since the delivery, I am not able to function properly. I always cry and unable to perform any of my duties, I need a solution"

"Will this survey help the medications be more available?!"

"we do not need surveys, we need actions, we need sewing, crafts,etc projects"

"I am a rural woman, my day is too busy, I do not have time to be depressed"

A patients friends said: "we are all depressed, if you come ask us or study us, you will be able to earn a Ph.D in depression"

CHAPTER 7

FISH AS SURROGATES FOR MAMMALS

There is an interesting similarity between fish and mammals in their genomes. The genomic correspondence between fish and humans led to the resemblance of the organ systems, physiological processes, and developmental pathways between fish and humans. Extrapolating data from fish models to humans helped broaden the understanding of the functions of human genes associated with abnormalities or diseases [13].

One major example of fish species that serve as surrogates for mammals in scientific research is the fathead minnows (Pimephales Promelas). It is a species of vertebrate fish, teleost, that belongs to the Cyprindae family. Fathead minnows contain major organ systems similar to those in humans, such as the reproductive system. The female reproductive system of the fathead minnows contain a bilateral ovary that produces mature eggs after they undergo maturation process consisting of six stages during which morphological changes occur [48].

Fathead minnows have been used extensively as a model for ecotoxicological and regulatory research. Data acquired after using the fathead minnow as a model for testing the toxicity of various chemicals can be successfully extrapolated to other species. For instance, toxicity data obtained from the fathead minnow are proven to be predictive to adverse, effects on other endangered species of fish [45].

Further, the validity of establishing cross-species as well as fish-mammals extrapolations depend on identifying toxicity pathways and how it is similar/different from the animal used in the experiments or the animal for which data are extrapolated to. Toxicity pathways are described by the initiation of an interaction of a compound with a biological molecule (e.g., hormone, enzyme) at the subcellular level leading to a cascade of events that alters the biochemical, physiological, and organ systems leading to adverse outcomes in the organism. Identifying the possibility of an interaction between a certain chemical compound-depending on its chemical structure- with a certain biological molecule can help in predicting

the resulting cascade of events and consequently the toxicity pathways in a wide array of organisms. Moreover, knowing the toxic mechanisms of action of chemicals help explain the toxicity pathways that could be similar in different organisms. For example, several studies investigated the mechanism of action of the endocrine disrupting chemical EDC [10]. It was found that some EDC bind to the androgen receptors in both fathead minnows and mammals. This shows the conservation of the hypothalamic-pituitary-gonadal axis between the fathead minnows and mammals. This emphasizes the validity of extrapolating toxicity data from fathead minnows models to mammals [47]. Another example, SSRI on fathead minnows showed a decrease in binding of serotonin to the serotonin reuptake transporter (SERT) [43], which is the same pharmacological effect and mechanism of action taking place in humans.

CHAPTER 8

FATHEAD MINNOW ENDOCRINOLOGY

8.1. HPG Axis

Mammals and teleost fish share the basic components of the HPG axis. Within the brain, the hypothalamus innervates secretory cells in the pituitary gland. In contrast to other vertebrates, the pituitary gland of the fish is less complex in structure. Also, in teleost, the innervations from the hypothalamus to the pituitary gland are more direct and the blood vessels connections within the pituitary gland compartments are more simple (i.e., no portal system connects the neurohemal neurohypophysis and the adenophysis, the two compartments of the pituitary gland) [32].

The hypothalamus releases gonadotropin-releasing hormone GnRH, which in turn stimulates the pituitary to release gonadotropins: follicle stimulating hormone (FSH) and luteinizing hormone (LH). The chemical structures of the FSH and LH in mammals and fish are similar, resulting in similar physiological and developmental processes. FSH is responsible for the follicle growth and development in the female ovaries and spermatogenesis in male testes. On the other hand, LH plays a role in the final steps of ovulation and spermiation [32].

Interestingly,

- FSH and LH are secreted from the proximal part of the pituitary gland, where plenty of the GnRH-immunoreactive fibers exist.
- There are three forms of GnRH. regulating the release of gonadotropins (FSH and LH) is their essential role. GnRH mediate their functions through five subtypes of re-ceptors. Research shows that GnRH 1 and 3 dominate in the pituitary gland, with a certain subtype of receptors mediating their functions on the pituitary gland.
- There is no evidence to that the release of the GnRH from the pituitary and conse-quently the release of the FHS and LH are pulsatile in fish as in mammals.
- Gonadal differentiation and consequently sex determination in fish depend on both

genes and environmental conditions [**32**].

8.2. Previous Studies with Endocrine Active Chemicals

Fathead minnows are used as one of the recommended species for testing for potential endocrine disrupting activity for chemicals [**6**].

Ankely et al. [**7**] studied the effects of ketoconazole KTC, a fungicidal agent, on the HPG axis on fathead minnows. KTC is a steroidogenesis inhibitor. It inhibits two major Cytochrome P450 genes involved in steroid synthesis in vertebrates: CYP11a (which is responsible for the first step in converting cholesterol into pregnenolone through side chain cleavage in the cholesterol molecule, [**31**]) and CYP17 (c17a-hydroxylase/17, 20-lyase). Results indicated that exposing adult fish to 30 PPB or 300 PPB KTC for 8 days period resulted in (a) a decrease in the levels of the testosterone (T) in males and females fathead minnow and a decrease in 17β- estradiol (E2) levels on females during exposure and recovery of ex vivo assays (b) in vivo results indicated that a decrease in the plasma levels of T and E2 occurred but the levels of the hormones rose again. (c) Despite in the up-regulation in the expression in the T and E2 genes in the gonads, the up-regulation did not explain the corresponding recovery in the hormones level. Ankely et al concluded that the difference between the ex vivo and in vivo recovery levels of hormones indicates that a complex compensatory mechanism is taking place in the HPG axis of the fathead minnows and following gene expression and the mechanism of action at the molecular levels can be indicative of potential endocrine activity of chemicals [**7**].

Villeneuve et al. [**45**] exposed female fathead minnows to fadrozole, aromatase inhibitor agent. Aromatase represents the rate limiting step in the conversion of testosterone (T) to 17β estradiol (E2). Fish were exposed to 3 and 30 PPB fadrozole for 8 days. Expressions of the key endocrine regulatory genes were analyzed early after exposure and at the end of the experiments. Plasma E2, vitellogenin, and ex vivo E2 levels were significantly decreased for both concentrations. However, recovery of the levels of the previous markers was evident after 1 day for the 30 PPB concentration and 8 day for the 3 PPB concentration. The recovery was accompanied by the increase in the gene expression of compensatory mech-

anisms, including aromatase (A isoform), and the protein involved in steroidogenesis e.g., CYP11A, acute steroidogenic regulatory protein StAR, and FSH receptor. Villeneuve et al. concluded that compensatory mechanisms in fish are evident and they are concentration and time dependent. Therefore, time and concentration of the chemicals should be considered on defining toxicity potential of chemicals [45].

17B-Trenbolone is a growth promoter that is used to enhance the growth of livestock. It is widely used in North America. The drug and its metabolites have androgenic characteristics, in addition to their chemical stability in the environment. Therefore, 17B-Trenbolone raised questions concerning its environmental safety [9]. Ankley at el. [9], tested the effects of exposing female and male fathead minnows to 17B-Trenbolone for 21 days at concentrations ranging from 0.005 PPB to 50 PPM. Female fish acquired masculine characteristics, including formation of the dorsal (nuptial) tubercules that are exclusive to male fathead minnows, and significant decrease in the levels of vitellogenin, 17β-estradiol, and testosterone occurred. On the other hand, at higher doses than the doses causing toxicity to females, males showed increase levels of vitellogenin and B-estradiol with a decrease in the level of ketotestosterone. Ankley at el concluded that 17B-Trenbolone represents a major fertility and reproductive toxicant to fish [9].

Ankley et al. [8] exposed the fathead minnows males and females to methoxychlor, a pesticide, at a concentration of 5 PPB and methyltestosterone at concentrations of 0.2 PPM. The period of the experiment was 21 days. Methoxychlor and its metabolites have an estrogenic activity on the estrogen receptor; therefore , they caused a reduction in the plasma levels of at least one of the following steroids (testosterone, 11 ketotestosterone, B-estradiol). Also, Methoxychlor increased the levels of vitellogenin in both females and males. Furthermore, methyltestosterone has androgenic activity. As a result, masculinization of female fathead took place, with the appearance of nuptial tubercles. Moreover, methyltestosterone caused alteration of the gonadal histology and weight in both sexes. Methyltestosterone caused aromatization of the androgen metabolites making them able to bind to the estrogen receptors causing unanticipated estrogenic stimulation of estrogen receptors which leads to

increase in the vitellogenin plasma levels in both sexes [8].

CHAPTER 9

SERTRALINE

9.1. Background of Sertraline

It is an antidepressant agent from the class of selective serotonin reuptake inhibitors (SSRI). It was introduced to the market by Pfizer incorporate in 1991. Sertraline is sold under the trade names of Zoloft $^®$, Altruline, Lustral, and Tatig [1].

9.2. Sertraline Distribution

Sertraline uptake in the brain tissue was higher than other antidepressant medications e.g., Buprobion. Sertraline have no effects on the morphology of the hapatocytes [39], despite the high Sertraline concentration found in the liver tissue [35].

9.3. Endocrine and Reproductive Effects of Sertraline and Other SSRI

Several studies investigated the pharmacological and toxicological aspects of sertraline. For example, Schultz et al. [39] investigated the effects of exposing male fathead minnows for sertraline for 21 days. Sertraline as well as Fluoxetine, which are both SSRI, caused alteration of testes morphology and physiology. However, the reproductive behavior was not affected nor spermatogenesis by neither drugs. In fact, increase in serotonin levels after exposing fish to SSRI enhances LH-induced effects through 5HT2-like receptors [42]. Norris [32], demonstrated that LH leads to increase in testosterone spermatogenesis in teleosts. Female zebrafish showed reduction in vitellogenin and 17B-estradiol when exposed to fluoxetine [26]. In contrast, fluoxetine showed estrogenic properties that led to significant increase in vitollogenin in male fathead fish at 28 ng/L concentration [39].

9.4. Sertraline Between Clinical Use and Scientific Research

A case study reported an increase of hot flashes for a woman in the reproductive age receiving fertility treatment while on SSRI. The case study identified that the effect of sertraline on reproduction and FSH levels is still unknown [40]. Another study indicated that sertraline was helpful in reducing psychological symptoms for women undergoing

gonadotropin-releasing hormone (GnRH) agonist as a treatment for endometriosis [46]; nevertheless, it was unclear whether or not sertraline affected the effectiveness of the GnRH agoinst agent. This leaves a gap in understanding of the effect of sertraline on the HPG axis in humans.

Previous studies reported androgenic effects of Fluoxetine on female zebra fish [26], and estrogenic effects on male fathead minnows [39]. Moreover, sertraline showed estrogenic effects on male fathead minnows.

9.5. Objectives

The lack of data about effects of sertraline in the hypothalamic-pituitary gonadal axis in female fathead minnows using FSH and LH endpoint, is needed. The results of this research will enhance the understanding of potential effects of the sertraline to female fathead minnows from the ecological point of view as well as the understanding of the possible hormonal effects of sertraline on human females taking the medication.

CHAPTER 10

METHODOLOGY

10.1. Test Organisms

Adult female fathead minnows approximately five months old were selected for this study. Fish were obtained from the Tarrant County Regional Water District. Fish were housed in dechlorinated tap water using a flow-through system. Temperature was kept at $25\pm 1\,°C$, with a 16:8 h light/dark cycles. Fish were fed twice daily with Tetramine flakes. Dechlorinated tap water was run in the flow-through system for one day prior to the start to flush the system of any residual chemicals.

Water quality parameters were measured before starting the experiment and at points during the experiment. pH, DO, alkalinity, hardness, ammonia, nitrate, nitrite, were within the normal ranges [33].

10.2. Experimental Design

The study was conducted for 7 days. A continuous flow-through diluter system in conjuction with a syringe pump was used deliver exposure solutions of sertraline (3 and 10 g /L) to fish. Dimethyl-formaldehyde (DMF) was used as the carrier solvent for the study with final concentrations in exposure water less than or equal to 0.01 %. Three 5-L tanks, each containing 6 fish, were used for the control and the two exposures.

10.3. Sertraline Preparation

A master stock solution of 10 mg/mL sertraline in methanol was prepared from sertaline hydrochloride (Sigma Aldrich, St. Louis, MO, USA). 27.8 and 8.34 mg/L substocks of sertraline in DMF were then prepared from the master stock solution. Substock solutions were delivered at a flow rate of 5 L/min to mixing chambers, each receiving a continuous supply of fresh water at a flow rate of 13.9 mL/min. Final dilution of these substocks achieved sertraline concentrations of 3 and 10 g/L in the exposure tanks. All fish tanks received four turn-overs of water per day. Solvent control tanks were Sertraline treatment exposures (3

and 10 ppb) were selected according to Huggett et al. [21] who indicated that effects in fish are similar to those in humans when fish are exposed to human plasma therapeutic levels. Moreover, at pH 8.5, 3 and 10 ppb yield internal plasma concentrations exceeding the human Cmax of sertraline (i.e., 142 ng/mL) at which pharmacological effect occurred [43].

10.4. Tissues Sampling

At day 7, fish were anesthetized using 1 ethyl 3-aminobenzoate methanesulfonate salt (MS-222, Sigma-Aldrich, St Louis, MO, USA) to obtain brain and ovary tissues. Range of ovaries weight is 1.3 to 84.9 mg. Tissues were kept in Liquid nitrogen (RNA Latter) and were snap-frozen for qRT-PCR analysis.

10.5. Real Time-Polymerase Chain Reaction (RT-PCR) Background

Real time-Polymerase Chain Reaction is widely used method to amplify and quantify double strands DNA, using primers that bind to genes of interest, enhancing building complementary templates to the gene of interest. However, reverse transcriptase precedes this process to ensure formation of complementary DNA (cDNA) strands from RNA strands containing the expressed gene that later will undergo PCR [25].

In order for the primers to anneal, single stranded cDNA has to be formed. This is achieved by raising the temperature to 95 °C at which DNA double strands denature. Upon cooling, the short, abundant, and kinetically active synthetic primers are faster than endogenous complementary DNA strands in defining and annealing to their target genes. After annealing the temperature is raised slightly to allow optimum conditions for the polymerase to start extending the DNA strand until reaching the 5 end. Further, the temperature is increased to the denaturation temperature allowing the previous cycles to be repeated, resulting in amplification of cDNA strands [25].

A unique feature of PCR is that the primer annealing to a DNA single strand is attached to another primer of opposing polarity. This allows the second primer to extend the complementary DNA strand formed by the original primer (amplicon). Hence, the amplification of DNA after each cycle is exponential (2N, where N represents the number of

cycles), Which results in enormous increase in the amount of the DNA of gene of interest with each cycle. As a result, 40 cycles of PCR produce enough amplification of an invisible gene to an adequate amount of DNA that is detectable by the fluorescent dye. Real-time PCR indicates that detection of the forrmed strands is instantaneous during their synthesis though measuring the change in fluorescence. Moreover, RT-PCR evaluates the number of cycles needed for a gene to produce a detectable fluorescent signal (threshold cycle, CT) [25].

10.6. FSH and LH Gene Expression Using RT-PCR

The expression of the steroidal genes, FSH and LH, were analyzed using real-time one-step RT-PCR assay. Rcorbett Rotor-Gene-6000 (Corbett Life Sciences, Mortlake, NSW, Australia) device with the Rotor-Gene 6000 Series Software version 1.7.40 were used. Following the manufacturers instructions detailed in the Rotor-Gene SYBR Green RT-PCR kit, a 72-well plate was loaded with 25 L of the reaction mix, containing 2.5 L of 40 ng/ L RNA, 5 L forward and reverse primers, 17.5 L of Mater Mix (12.5 L SYBR Green, 4.75 L RNAse free water, 0.25 Reverse transcriptase mix) (Corbett Robotics Inc., San Francisco, CA, USA).

RNA was extracted using TRI reagent from gonads and brain tissues then a suspension of RNA pellets with Tris-EDTA buffer was stored in $-80\,°C$. Before initiating any qRT-PCR run, RNA quality (RNA /Protein ratio) and quantity were assessed using BioTek Synergy 2 utilizing the Take Three Session feature on the Gen5 1.11 Software (BioTek, Winooski, VT, USA).

The samples were allowed to undergo reverse transcription for 10 minutes under $55\,°C$. followed by denaturation of double stranded DNA at $95\,°C$ for 5 minutes. Annealing of the primers and extension along DNA was achieved upon reducing the temperature to $60\,°C$ for 10 minutes. Raising the temperature to $95\,°C$ allow the cycle to be repeated. In this experiment, the cycles were repeated 40 times through thermo-regulation (Rotor-Gene SYBR Green RT-PCR kit).

10.7. Data Analysis

Data imported from RT-PCR device to Microsoft Excel spreadsheet were used to determine relative gene expression of FSH and LH genes using $2^{-\triangle\triangle CT}$ method as described by Schmittgen and Livak [27]. Target gene expression is normalized to an endogenous reference,

and compared relative to a calibrator. In this method, expression of the target genes in untreated control is used as calibrator genes. Ribosomal protein L8 gene (RPL8) was used as an internal reference gene. For each exposure, quantitative values of FSH and LH gene expression are normalized to RPL8 gene expression values from the same exposure concentration giving ($\triangle CT$). The value of each $\triangle CT$, was subtracted from ($\triangle CT$ mean) which represent the mean of the Ct values of FSH and LH from the solvent control group normalized to Ct values of RPL8 from the solvent control group. Thus, the resultant difference is the $\triangle\triangle CT$. For each exposure, ($\triangle\triangle CT$ mean) is calculated from averaging the values from each fish and its replicate and is used in equation: Amount of target gene = $2^{-\triangle\triangle CT}$ to obtain fold induction and amount of up or down regulation.

10.8. Calculating $\triangle\triangle CT$ for FSH

(1) From the solvent control group: Ct values for expression of calibrator genes (FSH or LH) were normalized using Ct values of RPL8 gene expressions. Average of the resultant Ct values was calculated ($\triangle CT$ mean).

(2) From RT-PCR data, Ct values for target gene expression FSH from the fish exposed to 3pp sertraline were acquired.

(3) Ct values of RBL8 expression in the fish exposed to 3 ppb sertraline were used to normalize Ct values from (2). The resulting Ct values are called $\triangle CT$

(4) $\triangle CT$ values were subtracted are subtracted from $\triangle CT$ mean calculated in step (1). The resultant values are called $\triangle\triangle CT$.

(5) Since RT-PCR causes exponential increase in amplicons, calculating fold induction is achieved from $2^{-\triangle\triangle CT}$

(6) This value is indicative for quantity of down regulation occurred for FSH gene in the gonads after exposure to 3 or 10 ppb sertraline for 7 days.

(7) Students T-test was used to detect the significance in the difference between Ct calculated in step (1) (result of normalizing calibrator genes FSH from solvent control tank and the endogenous control gene RPL8 from solvent control tank) and $\triangle CT$ values from step (3). A t-test less than 0.05 is considered significant, as two tailed, type two significance was set in the t-test equation in all the experiments.

(8) The same method was followed to calculate (6) for all the other unknowns: 10 ppb FSH gene expression, 3 ppb LH gene expression, 10 ppb Gene expression

CHAPTER 11

RESULTS

11.1. FSH and LH Expression in Gonad

11.1.1. RPL8 Standard Curve

RPL8 Gene expression from control samples (solvent control tank) was used as an endogenous control gene in all the experiments. In this experiment, we used the DNA of one fish from the control solvent control tank to prepare DNA serial dilutions. The Cycle Threshold CT values were plotted against the corresponding logarithmic (base 10) of the DNA concentration. Standard curve was built using linear regression Figure 11.1(R^2 0.99).
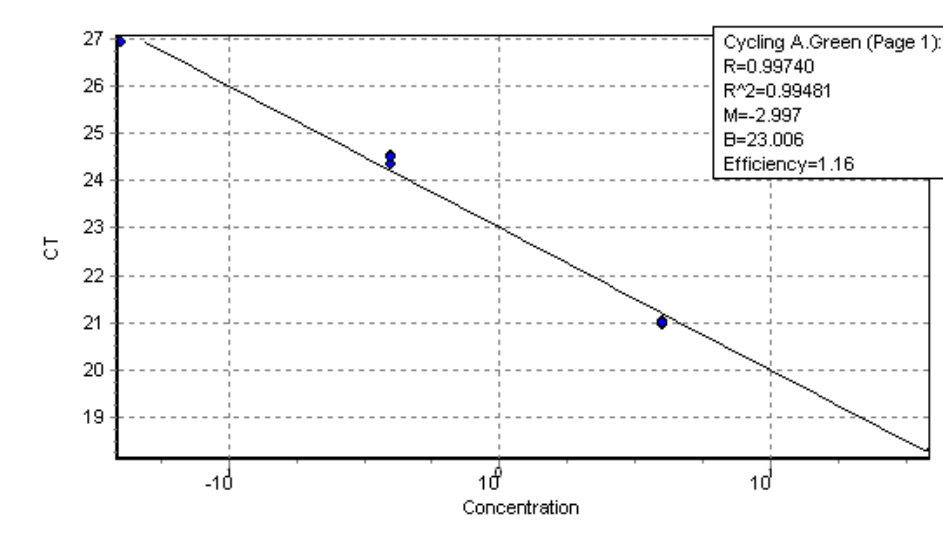

FIGURE 11.1. RPL8 Standard Curve

11.1.2. FSH Standard Curve

FSH gene expression from control samples (solvent control tank) served as a calibrator for relative gene expression of FSH in the exposure 1 and 2 tanks. Ct values were obtained from the software to be normalized by CT values of the endogenous control gene RBL8. The resultant Ct values serve as a reference to the FSH expression from exposure tanks. Logarithmic (base 10) of the concentrations of serial dilutions of concentrations from the

FSH from the control sample were plotted against the corresponding CT values. Standard curve was built using linear regression as shown in Figure 11.2(R^2 0.95).
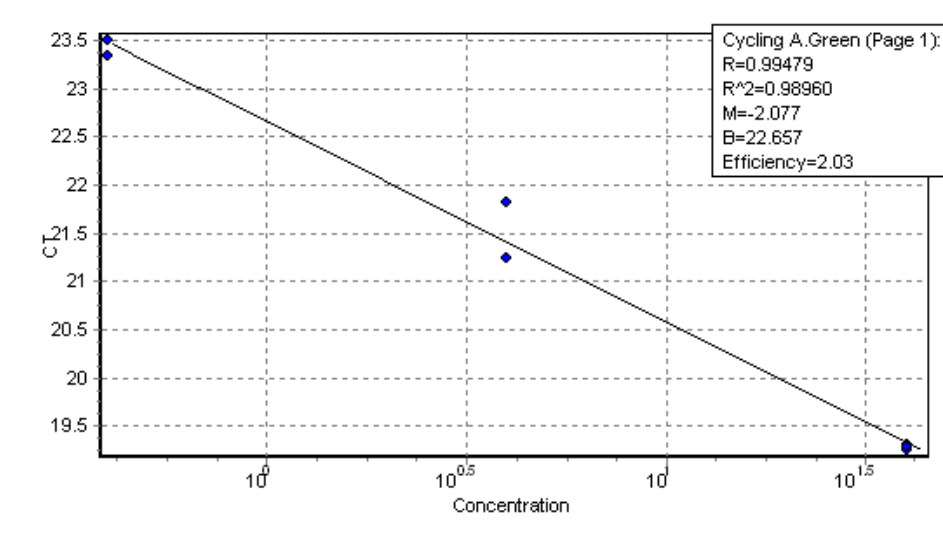

FIGURE 11.2. FSH Standard Curve

11.1.3. LH Standard Curve

LH gene expression from control samples (solvent control tank) served as a calibrator for relative gene expression of FSH in the exposure 1 and 2 tanks. Serial dilutions of LH from solvent control tanks were prepared. And the corresponding Ct values from those concentrations were obtained from the software to be normalized by CT values of the endogenous control gene RBL8. The resultant Ct values serve as a reference to the LH expression from exposure tanks. Logarithmic (base 10) of the concentrations of serial dilutions of concentrations from the DNA from one fish from the solvent control group were plotted against the corresponding CT values. Standard curve was built using linear regression Figure 11.3 (R^2 0.96).

11.2. Gene Expression of FSH and LH in Gonad

The results showed that a significant down regulation in FSH at the 3ppb exposure level (t-test 0.015) and in the 10 ppb (t-test 0.075). Also, a more pronounced decline in LH gene expression was found in the 10 ppb (t-test 4×10^-5) and the 3 ppb (t-test 0.01).Effects
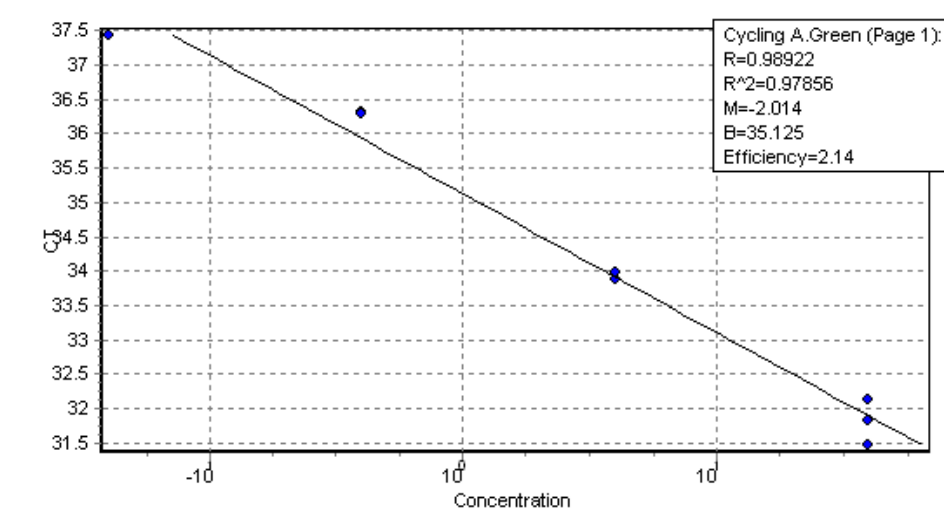
FIGURE 11.3. LH Standard Curve

of sertraline on gene expression of FSH and LH in the gonads as measured by $\log(2) \triangle\triangle CT$ is illustrated in Figure 11.4
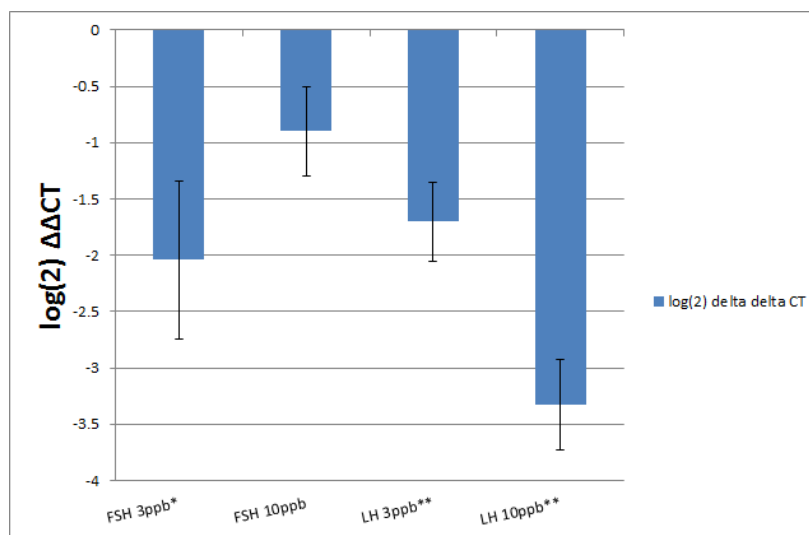

FIGURE 11.4. Gene Expression of FSH and LH in the Gonads

*On the gonads, the significance of the down regulation of FSH at the 3 ppb disappears after normalizing the FSH Ct values with Ct values of the RPL8 from the exposure level 3 ppb. Therefore, the results shown on FSH 3ppb are a calculated after normalizing FSH ct values to RPL8 values from the solvent control group.

** Results are significant when using the RPL8 values from either 3 ppb or solvent control group.

11.3. FSH and LH Expression in Brain

Results showed that a significant down regulation in LH in the 10 ppb (t-test 0.039). No significant up regulation was found in LH 3 ppb (t-test 0.82). However, a significant upregulation in FSH in 10 ppb (0.039) and upregulation on the borderline of significance on the FSH at 3 ppb (0.055) occurred after 7 day exposure to sertraline. Effects of sertraline on gene expression of FSH and LH in the brain as measured by log (2) $\triangle\triangle$ CT as demonstrated in Figure 11.5
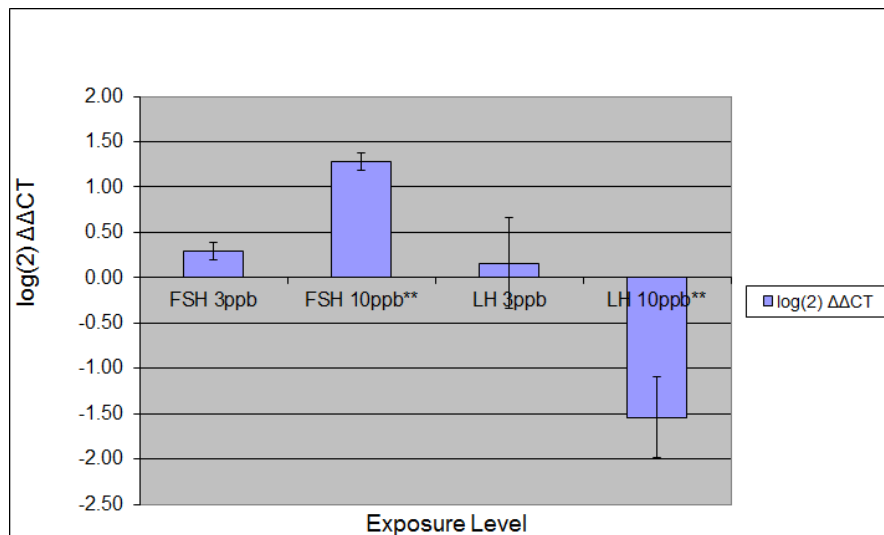

FIGURE 11.5. Gene Expression of FSH and LH in the Brain

Standard error of mean of the log (2) $\triangle\triangle$ CT was used to indicate how the log (2) $\triangle\triangle$ CT mean is widely distributed from the each log (2) $\triangle\triangle$CT value for each experiment.

CHAPTER 12

DISCUSSION

The results indicate that sertraline has a significant effect on the gene expression of the FSH and LH in the brain and the gonads. It can be inferred that sertraline causes up regulation to FSH on and LH (only at the lower concentration-3ppb) in the brain. However, the significant down regulation of all the genes and at all the concentrations on the gonads suggest that a negative feedback inhibition taking place, leading to up regulation for the estrogen levels.

Up regulating estrogen could be clinically a better effect for females than having a down regulation of estrogen. Accoring to Gordon and Speroff [17], in menopause, there are high levels of FSH and LH which leads to a decline in estrogen level that causes the following:

- Vasomotor symptoms: decline in estrogen level is suggested to be the reason for hot flushes; the hallmark of menopause.

- Genitourinary atrophy: urethritis, urgency incontinence, and urinary frequency. The function of the urinary tract can take up to 6 to 12 months of treatment.

- Insomnia

- Cardiovascular disease: the decline in estrogen predispose women to CVD as a result of depriving women of the protective mechanisms of estrogen, such as

(1) Increase in HDL and decrease in total cholesterol and LDL

(2) Anti atherosclerotic effect on the arteries

(3) Helping protect the endothelial cells from injury

- Osteoporosis: reduction of estrogen leads to development of osteoporosis because estrogen has protective role in the bones through:

(1) Supporting osteoblasts and prevents the distortion of the osteobalst:osteoclast balance.

(2) Augmentation of vitamin D receptors. As a result, enhancing calcium absorption from the GIT [17].

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In normal females, FSH and LH levels have certain levels throughout the menstrual cycle. These levels are important for conserving the proliferative, ovulatory, and secretory phases of the menstrual cycle. For instance, FSH levels are high during the early follicular phase where FSH main function is to develop the follicle. Examining serum level of FSH on day 2 and 3 along with other factors e.g., estradiol level, and patients age help clinicians determine the oocyte quality and possibility of successful conception in assisted reproductive technologies [44].

It is important to consider the amount of increase in estrogen. FSH is extremely sensitive to estrogen, as a result release of any amount of estrogen leads to inhibition of FSH. On the other hand, estrogen increases the release of LH. At levels of 200pg/ml, LH surge occurs [14].

Not only it is important to consider the alterations in the levels of FSH and LH that is caused by sertraline, but also it is essential to monitor the FSH:LH ratio. The FSH:LH ratio serves as an method for detecting responsiveness of the ovary to in-vitro fertilization and consequently pregnancy rates. The ovaries of women with FSH:LH ratio above 3 are more likely to have lower pregnancy success rates. Examining the FSH:LH ratio in women who have low LH and relatively normal FSH is useful for clinicians to adjust their treatment protocol to this group of patients [41].

GnRH is responsible for regulating the release of FSH and LH. GnRH is inhibited by serotonin and Dopamine [14]. Since Valenti et al. [43] showed that at the 3 and 10 ppb pharmacological effect is taking place (decrease in binding to SERT). Therefore, the significant down regulation shown in this study is suggested to be because of the inhibitory effect of serotonin. So based on peer reviewed literature blockade or inhibiting SERT will increase levels of serotonin in the synaptic cleft. These increased levels will alter GnRH and thus LH and FSH.

12.1. Limitation

Three fish were found to have a relatively low RNA / Protein ratio of approximately 1.55:1.65.

CHAPTER 13

CONCLUSION

Exposing fathead minnows to sertraline for 7 days resulted in a significant decrease in LH and to a lesser extent FSH. The down-regulation was evident in the 10 ppb concentration than at the 3 ppb. Our results suggest that LH and FSH are down-regulated as a result of inhibition of GnRH as an effect of increased levels of serotonin after blocking serotonin reuptake by sertraline.

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