Levonorgestrel exposure impacts the spermatogenesis in adult male frogs (Xenopus tropicalis)

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# Table of contents

Acknowledgements.................................................................................. 2  
Abstract.................................................................................................. 3  
1. Introduction.......................................................................................... 3  
  1.1 Background..................................................................................... 3  
  1.2 Spermatogenesis............................................................................. 5  
  1.3 Levonorgestrel (LNG)....................................................................... 6  
  1.4 Xenopus tropicalis.......................................................................... 7  
  1.5 Objectives....................................................................................... 7  
2. Material and Methods.......................................................................... 7  
  2.1 Animals and exposure..................................................................... 7  
  2.2 Fertility study.................................................................................. 8  
  2.3 Dissection and morphometry.......................................................... 8  
  2.4 Sperm count and motility................................................................. 8  
  2.5 Testicular histomorphometry............................................................ 9  
  2.6 Chemical analysis and water quality.............................................. 9  
  2.7 Statistics.......................................................................................... 10  
3. Results................................................................................................ 10  
  3.1 Health status................................................................................... 10  
  3.2 Fertility assessment......................................................................... 10  
  3.3 Sperm analysis................................................................................ 10  
  3.4 Testicular morphology..................................................................... 10  
  3.5 Testicular histomorphology............................................................. 11  
4. Discussion............................................................................................ 13  
  4.1 Conclusion...................................................................................... 14  
5. References............................................................................................ 15
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Abstract
Several pharmaceuticals are today regarded as environmental pollutants. They are found in aquatic environments all around the world. Levonorgestrel (LNG) is a synthetic progesterone found at low concentrations in sewage effluent and surface waters. It has been found to inhibit reproduction in aquatic vertebrates e.g. frogs exposed as tadpoles and fish. Developmental exposure to LNG resulted in sterile female frogs whereas no effects on male fertility were noted. What is the susceptibility of adult male frogs to progestin toxicity? The aim of this study was to investigate effects of LNG on the male reproductive system including spermatogenesis and sperm quality. The frogs were exposed to LNG for 28 days at the concentrations 0, 0.1 or 1 nM. Histomorphometrical assessments of the testes of LNG exposed frogs revealed an increase in the proportion of immature germ cells, spermatogonia, and a decrease in the number of seminiferous tubules/ testis. To the best of my knowledge this is the first study of reproductive toxicity of a progestin conducted on adult frogs. The results indicate that spermatogenesis seems to be a sensitive target for progestins in adult males.

1. Introduction
Amphibians are declining worldwide, and several factors are likely implicated in these declines including habitat destruction and environmental pollutants e.g. endocrine disrupting chemicals (EDs) (Houlahan et al., 2000). Focus has recently turned to the synthetic progesterone levonorgestrel (LNG), which is commonly used in contraceptives and has been detected in the aquatic environment. Recent research shows that LNG may pose a threat to aquatic wildlife as environmental LNG concentrations have been shown to inhibit egglaying in fish (Zeilinger et al., 2009). Information on reproductive toxicity of LNG in frogs is scarce but a study conducted on frog tadpoles showed that of LNG is a potent developmental toxicant causing female sterility (Kvarnryd et al. 2011).

1.1 Background
The attention to pharmaceuticals in the environment has increased over the past years (Fick et al., 2010). Pharmaceutical substances have been detected in surface water around the world and are today regarded as environmental pollutants. Pharmaceuticals are of interest because they are intended to be bioavailable and persistent enough to bring about a specific effect. These physico-chemical properties i.e. persistence and lipophilicity can lead to
bioaccumulation in the environment which might lead to negative impacts on entire ecosystems (Halling-Sørensen et al., 1998). Sources of pharmaceuticals in the environment are both human and veterinary medicines (Nikolaou et al., 2007). Concentrations (0.001-1 μg L\(^{-1}\)) of pharmaceuticals have been detected in sewage effluent and surface water (Fick et al., 2010). Aquatic organisms living in recipients of sewage treatment effluents may be especially vulnerable because they are chronically exposed to anthropogenic chemicals in the water. The main sink for chemicals in the environment is surface water, making studies on the effects of environmental contaminants on aquatic organisms very important.

The endocrine system is responsible for production and regulation of hormones and their receptors which in turn manage various physiological processes, such as reproduction and development. EDs can alter the function of the endocrine system by e.g. binding to the sex steroid receptors, mimicking or inhibiting actions of endogenous hormones (e.g. sex steroids). An ED that has attracted a lot of attention for the last ten years is the synthetic estrogen, ethynylestradiol (EE\(_2\)), commonly used in contraceptive pills. The estrogenic activity of EDs is suspected to be involved in reproductive disorders and developmental defects in wildlife (Guilliette, 1994; Toft et al., 2004). Studies conducted on wild riverine fish (\textit{Rutilus rutilus}) showed sexual and reproductive disruptions (Jobling et al., 1998; Jobling et al., 2002). The fish living near sewage effluent known to contain estrogenic chemicals showed a high incidence of intersex males with both male and female gonadal tissue (Jobling et al., 1998). Other aquatic vertebrates such as amphibians are also very susceptible to endocrine disruption. Laboratory experiments conducted on frogs (\textit{Xenopus tropicalis}) showed disruption of the testicular and oviductal development resulting in reduced male and female fertility after larval exposure to EE2 concentrations found in surface waters (Gyllenhammar et al., 2009).

Recent research has focused on other hormones used in contraceptive pills. Synthetic progesterone (progestin), is used for instance in different contraceptive pharmaceuticals and in post-menopausal therapy medicines (homepage of Fass). Progesterone is a sex steroid hormone which is important for male and female reproduction. Progesterone receptors (PR) are expressed throughout the male and female reproductive organs including the testicle. In male vertebrates progesterone plays a vital role in sperm motility and sperm maturation. Progesterone action is regulated via the hypothalamus-pituitary-gonad axis (HPG). The hypothalamus releases gonadotropin-releasing hormone (GnRH) that stimulates the pituitary
to secrete the luteinizing hormone (LH) and follicle-stimulating hormone (FSH). LH and FSH act on the gonads leading to synthesis and release of sex steroids e.g. progesterone.

Progestins mimics progesterone to some extent and interact with the PR. The main contraceptive actions are; inhibition of ovulation, production of a thick cervical mucus and transformation of endometrium making it unsuitable for implantation (Erkkola and Landgren, 2005). The progestin levonorgestrel (LNG) is common in contraceptives and it has been detected in the aquatic environment. LNG concentrations in blood plasma of fish caged downstream sewage treatment plants in Sweden ranged between 8.5 and 12 ng mL\(^{-1}\) (Fick et al., 2010). These levels exceeded the LNG concentrations (1 ng L\(^{-1}\)) in the sewage effluent showing that LNG is readily bioconcentrated (Fick et al., 2010). Female fish exposed to 0.8, 3.3 or 29.6 ng LNG /L had inhibited egg laying and at the highest concentration they displayed signs of masculinization (Zeilinger et al., 2009). These findings show that progestins may pose a threat to reproduction in wild fish. A recent study on frogs shows that tadpole exposure to LNG severely impaired oviduct and ovary development. LNG concentrations of 19 ng/ L (0.06 nM) and 158 ng /L (0.5 nM) caused oviductal agenesis, inhibited oocyte maturation and sterility in female *X. tropicalis* whereas no effects could be observed on testicular development and fertility in males (Kvarnryd et al., 2011).

1.2 Spermatogenesis

Spermatogenesis is the process during which mature sperm cells develops from germ cells. Spermatogenesis in amphibians (frogs and toads) is similar to that of teleost fish (Pudney, 1995). The testis of frogs (and several fish species) consists of seminiferous tubules with germ cell nests in different maturation stages. Within each nest, the germ cells develop synchronously. The nests are produced when a sertoli cell connects to a primary spermatogonium. The spermatogonium enters mitosis and produces a cluster of secondary spermatogonia which is enclosed by the sertoli cell (Pudney, 1995). Secondary spermatogonia transform into primary spermatocytes which undergo meiotic division into secondary spermatocytes. Transformation then proceeds into round and elongated spermatids and finally to spermatozoa (Fig. 1). The last step in spermatogenesis is spermiation, that is when the nest wall breaks and spermatozoa move into the lumen of the seminiferous tubule (Pudney, 1995). The process of spermatogenesis is complex and depends on hormonal and local controlling mechanisms which is not yet fully understood (Pierantoni et al., 2002; Sasso-Cerri et al., 2004).
Figure 1. Segments of the different stages of germ cell nests in a seminiferous tubule during spermatogenesis in zebra fish testis. The germinal epithelium contains Sertoli (SE) and germ cells. The interstitial Leydig cells (LE), blood vessels (BV) and peritubular myoid cells (MY) are shown. Type A undifferentiated* spermatogonia (Aund*); type A undifferentiated spermatogonia (Aund); type A differentiated spermatogonia (Adiff); spermatogonia type B [B (early–late)]; leptotenic/zygotenic primary spermatocytes (L/Z); pachytenic primary spermatocytes (P); diplotenic spermatocytes/metaphase I (D/MI); secondary spermatocytes/metaphase II (S/MII); early (E1), intermediate (E2) and final spermatids (E3); spermatozoa (SPZ) (Schulz et al., 2009)

1.3 Levonorgestrel (LNG)

Synthetic progesterone is developed from either testosterone or progesterone. LNG is derived from testosterone and is included in the gonane group of 19-nortestosterone derivatives (Sitruk-Ware, 2008). LNG binds to the receptors of progesterone, androgen and estrogen with the binding affinity of 323, 58 and < 0.02 %, respectively, compared to the endogenous human hormones affinity for their target receptor, which is set to 100%. The chemical name of LNG is 13-Etyl-17-hydroxi-18, 19-dinor-17α-pregn-4-en-20-yn-3-on. LNG has a molecular weight of 312.446 g mol⁻¹, a Log $P_{ow}$ of 3.55 and CAS no. 797-63-7. The molecular structure is shown in Fig. 2.

Figure 2. The molecular structure of levonorgestrel (Homepage of Fass).
1.4 Xenopus tropicalis

The model organism used in this study is the Western clawed frog (*Xenopus tropicalis*). It is related to the South African clawed frog (*Xenopus laevis*) which is a common laboratory animal. Some advantages of *X. tropicalis* are its lesser size and shorter generation time than *X. laevis*. *X. tropicalis* are native to Africa and their habitat is commonly muddy ponds. An adult female *X. tropicalis* has a body size of 4-5 cm from snout to vent and a weight of 10-50 grams. Males are generally smaller than the females. Sexually mature *Xenopus* males have darkened nuptial pads on their forelimbs and females have a large protruding cloaca. They are fully aquatic during their whole life cycle, which makes them suitable for aquatic studies.

1.5 Objectives

The main objective of this study was to investigate effects of LNG on fertility and reproductive organs of adult male *X. tropicalis*. Male frogs were exposed to LNG via the water for 28 days. After exposure they were mated with unexposed females to evaluate fertility success. Sperm quality and spermatogenesis were evaluated using cytological and histomorphometrical analyses. A second objective was to contribute to the development of methods to study reproductive toxicity in male frogs.

2. Materials and Methods

2.1 Animals and exposure

Eight adult male frogs (*X. tropicalis*), (Xenopus 1, Dexter, MI, USA) were exposed to LNG (CAS no. 797-63-7., Sigma-Aldrich., Steinheim, Germany, purity ≥ 99%) for 28 days. The frogs were exposed separately in 15 L plastic tanks to 0, 0.1 and 1 nM LNG, 31.6 ng/ L and 316 ng/ L, respectively (nominal concentrations). The exposure system was semi-static in which half of the water volume (7.5 L) was renewed and new test solution (15µl) added daily. The water consisted of seven parts deionised water and three parts copper free tap water. The number of frogs in each exposure group was, 3, 3 and 2 respectively. The tanks were saturated two days prior to exposure. Acetone was used to dissolve LNG and the water in all tanks had an acetone concentration of 0.0002%. Samples of water for chemical analysis were taken on exposure day 7, before and after water change, to determine the mean measured concentrations on LNG in the tanks. The tanks were placed in random order in a water bath which held a temperature of 27 ±1°C. The water conductivity was 130-150 S/m. The frogs were fed tropical fish food (Excel Aquatic Nature, Sweden) three times a week. The light
period was 12:12 hour light-dark cycle. The animal experiments were conducted with approval from the Uppsala local ethics committee.

2.2 Fertility study
On exposure day 28 the males were mated with unexposed adult female frogs (X. tropicalis). Before mating the females were primed with human chorionic gonadotropin (hCG) for maturation of oocytes and activation of ovulation. Twenty international units (IU) of hCG in 100µl in 0.9% NaCl were injected into the dorsal lymph sac 24 hours before mating. Another 100 IU (100µl in 0.9% NaCl) was injected shortly before mating. The males were not treated with hCG in case it would interfere with the effects of LNG. The intention was that the hCG treated females would trigger the males into mating. Each frog couple was placed in a breeding tank (10 L) equipped with two petri dishes at the bottom, covered with a steel net for collection and protection of the eggs. The tanks were placed in a water bath with a temperature of 27±1°C and were covered with black plastic bags in a quiet room to mimic a safe reproduction environment.

The frogs were observed every 45 minutes to record timing of amplexus. After six hours the frogs were separated. The total number of ovulated eggs was estimated. The eggs were left in the tank for 24 hours after which fertilization rate was recorded. After the mating the males were anesthetized in 0.7 % benzocaine dissolved in 70% ethanol (CAS no. 94-09-7, Sigma-Aldrich, St. Louise, MO, USA) and killed by decapitation.

2.3 Dissection and morphometry
Reproductive organs were dissected after the frogs had been euthanized. Body weight, left testis weight and cloaca length were recorded. Gonadosomatic index (GSI) was calculated as left testis weight x 100/ body weight. The right testis with attached kidney was dissected and fixed in formaldehyde (4% in phosphate buffer) and processed for histological evaluation. The left testis was used for sperm analysis.

2.4 Sperm count and motility
The left testis was, directly after dissection, put in a beaker with a drop of Simplified amphibian Ringers solution (113 mM NaCl, 1 mM CaCl$_2$, 2 mM KCl, and 3.6 mM NaHCO$_3$). The testis was weighed and transferred to an eppendorf tube containing Ringers solution (0.01 mL/ mg testis weight). The sample was minced and centrifuged for two minutes to separate the spermatozoa from most of the surrounding tissue. 50 µl of the supernatant was transferred
to a tube containing deionised water (1:6 dilution) for activation of the spermatozoa. The sample was directly thereafter transferred to a haemocytometer (Fuchs-Rosenthal) and spermatozoa were recorded using a microscope (Leitz, Laborlux 12, x40). Motile and non-motile spermatozoa in eight squares were counted. Any sign of movement was scored as motility. The percentage of motile spermatozoa was calculated. Sperm concentration was determined as the number of sperm cells/ mg testis weight.

2.5 Testicular histomorphometry

The right testis connected to the kidney (to preserve vasa efferentia) was, after fixation, dehydrated in increasing concentrations of ethanol (70 – 99.5%) and embedded in methacrylateresin (Technovit 7100, Histolab, Sweden). Transverse sections (2µm) of the testis-kidney complex were sliced at three different levels (anterior – posterior). The sections were stained with hematoxylin (Mayer) in a microwave oven at 60°C for 7 minutes. Thereafter they were rinsed in tap water and stained with eosin (Y) for 1 minute at room temperature. The sections were analysed using a microscope (Leitz, Laborlux 12). The section from the most central part of the testis was used for the histomorphometrical assessment. The number of seminiferous tubules per testicular cross section was counted. Five seminiferous tubule cross sections were randomly chosen for analysis. Only tubular cross sections that had a fairly round shape and contained spermatozoa in lumen were analysed. Each of the five seminiferous tubules was assessed for the following histological parameters: diameter, number of germ cell nests, the stage of the germ cell nests, and amount of spermatozoa in lumen. The germ cell nests were classified into four stages; spermatogonia, spermatocytes, spermatids and spermatozoa according to the criteria defined by Kalt (Kalt, 1976). The amount of spermatozoa in the seminiferous tubules lumen was estimated and converted into the scores 1 to 3. Tubules with no or little spermatozoa were assigned score 1 and those with the highest amount were assigned 3. The diameter of the testis and seminiferous tubules was measured with the software Photoshop.

2.6 Chemical analysis

Chemical analysis of the water showed that the mean measured concentrations (mean, S.D.) of LNG were 0, 0.15 nM (±0.02) and 1.7 nMs (±0.07) corresponding to 47.4 ng/ L and 537.2 ng/ L. Chemical analysis of the water was conducted using liquid chromatography and mass spectrometry (LC/MS/MS) by Dr. Jerker Fick, Department of Chemistry, Umeå University, Sweden. Details of the analytical method are presented in (Kvarnryd et al., 2011).
2.7 Statistics

The results were analyzed for significant differences between the three exposure groups using Kruskal-Wallis-test (Graphpad Prism 5.0). T-test was used to analyze significant differences between the control and the exposed groups (0 vs. 0.1nM, and 0 vs. 1.0 nM). The level of significance was set to $p < 0.05$.

3. Results

3.1 Health status

There were no behavioural or eating habit anomalies or other signs of distress among the frogs. No deaths occurred.

3.2 Fertility assessment

Results from the fertility and reproduction studies are shown in Table 1. None of the couples including control animals formed amplexus. The fertility success of the males could therefore not be determined. All females except one that was mated with a male from the 0.1 nM exposure group laid eggs but there were no signs that the eggs were fertilized.

3.3 Sperm analysis

The motility of spermatozoa did not differ significantly between the groups. The highest frequency of mobile spermatozoa was seen in the 1 nM group (Table 1). The concentration of spermatozoa was lower in the exposure groups than in the control, but a statistical difference could not be ascertained.

3.4 Testicular morphology

There were no significant differences between the groups in GSI, testis weight or body weight (Table 1). The testis of one frog in the 0.1 nM exposure group and one frog in the control group were divided into three and two segments, respectively.
Table 1. Results from the fertility and reproduction study in adult male frogs (Xenopus tropicalis) after 28 days of exposure to levonorgestrel (LNG). The data are presented as mean (±S.D.) values, except for amplexus success.

<table>
<thead>
<tr>
<th>Concentration (nM LNG)</th>
<th>Amplexus success (%)</th>
<th>Body weight (g)</th>
<th>Testis weight (g)</th>
<th>GSI(^a) (%)</th>
<th>Concentration spermatozoa(^b) (10(^3))</th>
<th>Motile spermatozoa (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=3)</td>
<td>0</td>
<td>8.80 (0.36)</td>
<td>0.0141 (0.004)</td>
<td>0.160 (0.049)</td>
<td>647 (74)</td>
<td>49 (17)</td>
</tr>
<tr>
<td>0.1 (n=3)</td>
<td>0</td>
<td>8.78 (0.38)</td>
<td>0.0145 (0.002)</td>
<td>0.165 (0.019)</td>
<td>506 (191)</td>
<td>49 (11)</td>
</tr>
<tr>
<td>1.0 (n=2)</td>
<td>0</td>
<td>9.10 (0.37)</td>
<td>0.0159 (0.0006)</td>
<td>0.174 (0.0002)</td>
<td>263 (186)</td>
<td>71 (3)</td>
</tr>
</tbody>
</table>

\(n=\) Number of frogs
\(^a\) Gonadosomatic index, calculated as testis weight / bodyweight
\(^b\) Number of spermatozoa / mg testis

3.5 Testicular histomorphometry

The results of the histomorphometrical analysis are presented in Table 2 and 3. The LNG exposed groups had a higher fraction of spermatogonial nests compared to the control group (Fig. 3), but a statistically ascertained difference was found only between the 0.1 nM group and control (\(p=0.041\)) (Table 3). There were no significant differences between the groups regarding the proportions of the other germ cell stages \(i.e.\) spermatocytes and spermatids. The estimated amount spermatozoa in the seminiferous tubule lumen did not differ between the exposures.

Table 2. Results of histomorphometrical assessments of adult male frog (Xenopus tropicalis) testis after 28 days exposure to levonorgestrel (LNG). Data presented as mean (±S.D.)

<table>
<thead>
<tr>
<th>Concentration (nM LNG)</th>
<th>Cell nests/ seminiferous tubules (number)</th>
<th>Stages of germ cell nests in seminiferous tubules (%)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Spermatogonia</td>
<td>Spermatocytes</td>
</tr>
<tr>
<td>Control (n = 3)</td>
<td>13.7 (6.1)</td>
<td>8.4 (3.0)</td>
<td>66.2 (5.3)</td>
</tr>
<tr>
<td>0.1 (n = 3)</td>
<td>13.9 (5.3)</td>
<td>19.3 (5.6)*</td>
<td>57.9 (4.3)</td>
</tr>
<tr>
<td>1.0 (n = 2)</td>
<td>17.3 (5.8)</td>
<td>16.4 (7.9)</td>
<td>61.3 (2.5)</td>
</tr>
</tbody>
</table>

\(^*\) Significantly different compared to control \(p=0.041\)
Figure 3. The proportion of spermatogonial nests in testicular cross sections from male frogs (*Xenopus tropicalis*) after 28 days of exposure to levonorgestrel (LNG). Data presented as mean ± S.D. n=3, 3, and 2 in the control, 0.1 nM, and 1.0 nM, respectively. Significant differences were found between the three treatments. Statistics were examined by ANOVA, Kruskal-Wallis test (*p* = 0.025). * T-test, Mann-Whitney showed significant differences between control and 1 nM LNG *p* = 0.019.

The number of seminiferous tubules in the testis differed significantly between the three groups (*p* = 0.044, Table 3, Fig. 4). Males exposed to 1.0 nM LNG had a significantly reduced amount of seminiferous tubules per cross section compared to controls. No significant differences between the three groups in testis or seminiferous tubules diameter were found.

Table 3. Results of histomorphometrical assessments of adult male frogs (*Xenopus tropicalis*) testis after 28 days of exposure to levonorgestrel (LNG). The data are presented as mean (±S.D.) values.

<table>
<thead>
<tr>
<th>Concentration (nM LNG)</th>
<th>Testis diameter (mm)</th>
<th>Seminiferous tubule diameter (mm)</th>
<th>Seminiferous tubules/ testis (number per cross sections)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 3)</td>
<td>1.78 (0.45)</td>
<td>0.25 (0.063)</td>
<td>56 (5)</td>
</tr>
<tr>
<td>0.1 (n = 3)</td>
<td>1.62 (0.15)</td>
<td>0.26 (0.069)</td>
<td>43 (3)</td>
</tr>
<tr>
<td>1.0 (n = 2)</td>
<td>1.57 (0.70)</td>
<td>0.25 (0.054)</td>
<td>31 (12)*</td>
</tr>
</tbody>
</table>

* Significantly different from control group, *p* = 0.044.
Figure 4. The number of seminiferous tubules per testicular cross section from adult male frogs (*Xenopus tropicalis*) after 28 days of exposure to levonorgestrel (LNG). Data presented as mean ± S.D., n=3, 3, and 2 in the control, 0.1 nM, and 1.0 nM, respectively. * Significantly different from control group (p < 0.05).

4. Discussion

The results of this study showed that 31.6 ng/L (0.1 nM) of the progestin LNG induced an increase of the immature germ cells, spermatogonia, indicating that the spermatogenesis was impacted. To the best of my knowledge this is the first study of reproductive toxicity of a progestin conducted on adult frogs. A significant decreased number of seminiferous tubules was found at 316 ng/L (1.0 nM) LNG compared to the control. A preliminary finding in this study also indicated a lower sperm concentration in the LNG exposed males compared with the controls. Even though this difference was not statistically ascertained, the finding is interesting and will be followed up. Interestingly, exposure of *X. tropicalis* tadpoles to LNG at concentrations of 19 ng/L (0.06 nM) and 158 ng/L (0.5 nM) had no observed effects on testicular development, fertility, or sperm count in adult males (Kvarnryd *et al.*, 2011). This implies that male frogs are more sensitive to LNG as adults than as tadpoles.

In humans, progestin contraceptives act by suppressing gonadotropin (FSH and LH) secretion (Nieschlag *et al.*, 2003). An in vitro study conducted on testicular tissue from newts (*Cynops pyrrhogaster*) showed that FSH is needed for the differentiation of spermatogonia into spermatocytes (Yazawa *et al.*, 2002). In testicular tissue cultured without FSH the differentiation of spermatogonia into spermatocytes had completely ceased after two weeks, but the
proliferative activity of spermatogonia was still maintained, though at a decreased rate (Yazawa et al., 2002). If LNG caused a suppression of gonadotropin release in the exposed frogs this might explain the increased proportion of spermatogonia seen in the present study.

LNG is derived from testosterone and has partial affinity to the androgen receptor. Hence, it is interesting to compare the present impact on spermatogenesis of LNG with that of an androgenic substance. Exposure of adult male frogs (Rana pipiens) to the androgen dihydrotestosterone (DHT) for 30 days resulted in a reduction of spermatogonia and primary spermatocytes, while secondary spermatocytes, spermatids and spermatozoa were unaffected or stimulated by DHT (Tsai et al., 2005). These findings are directly contradictory to the result in the present study. This strongly suggests that the observed effects of LNG on spermatogenesis in adult X. tropicalis were progestagenic.

It was not possible to analyze fertility success of the LNG exposed males as the frogs failed to form amplexus when only the females were primed with hCG. The fertility success of the LNG exposed males must therefore be assessed by other endpoints such as spermatogenesis. The results of the present study indicate that spermatogenesis is a sensitive endpoint for progestagenic effects in male frogs. The indication of reduced sperm concentration in the LNG exposed males need to be followed up in order to understand the risk progestins may pose to fertility in male frogs.

The decreased number of seminiferous tubule in the testes of the LNG exposed males is difficult to interpret. No effects on testis size, weight, or seminiferous tubule diameter could be seen. In a 21-day study on adult fathead minnows (Pimephales promelas) males exposure to 0.8, 3.3 and 29.6 ng LNG/ L appeared to increase the testis and tubule area as well as the number of spermatozoa in the tubular lumen though the data was not statistically analyzed due to a low sample size (Zeilinger et al., 2009). The observations in the fathead minnows seem to be contradictory to the results in the present study.

4.1 Conclusions

Exposure of adult male frogs to LNG impacted the spermatogenesis by increasing the proportion of spermatogonia and decreasing the number of seminiferous tubule/ testis. Effects on spermatogenesis in frogs seem to be a sensitive endpoint for progestins. Further investigation into the reproductive toxicity of LNG in male frogs is warranted to understand the risk posed by progestagenic chemicals on reproduction in wild frogs.
5. References


