1 Introduction

1.1 Purpose

Using mouse and bovine models, this Spotlight focuses on the (a) methodology and (b) diagnostic value of two new imaging technologies in order to visualize the physiology and pathology of the male and female genital tracts under *in vivo* conditions. These methods have been established for their effective use in reproductive medicine by our research team. The following techniques will be covered: (a) digital videomicroscopy and (b) probe-based confocal laser endomicroscopy (pCLE). The aim of the book is to enable researchers to apply these techniques of *in vivo* microscopy in order to develop new insights into the effects of diseases or drugs on the functional integrity of the male and female genital tracts. Additionally, this book aims to clear the way for the establishment of routine use of pCLE in the daily practice in gynecology and andrology.

1.2 Establishing new techniques for *in vivo* microscopy in the male genital tract

In Europe, nearly every sixth couple of reproductive age is involuntarily childless. In about 50% of these couples, this is due to male infertility.[1] To date, routine infertility imaging of the testis and epididymis is based on ultrasonography. However, this technique lacks the resolution to reveal detailed morphology of the testicular tubules and the epididymal duct, which may be altered by urogenital infections, hypogonadism, or varicocele (the most frequent causes of infertility).[1] In addition, ultrasonography cannot visualize single spermatozoa, which are only 60 μm (human) to 80 μm (most mammals) in size.[2] In about 10% of infertile men, azoospermia is diagnosed, i.e., there are no spermatozoa in the ejaculate.[3] In this case, testicular sperm extraction (TESE) is performed, in which sperm is retrieved by cutting several multifocal tissue samples out of the testis. The localization of the biopsies is determined arbitrarily by the surgeon because there are no diagnostic tools to precisely localize vital spermatozoa in the testis. Thus, successful sperm retrieval is achieved in only 50% of the patients.[4] Success rates are further compromised by the fact that spermatogenesis is not homogenous and is clearly different in various locations.[5] Note that the highly invasive surgery during TESE includes a risk of inducing hypogonadism in about 7.5% of the patients.[6] This danger points to the necessity of establishing new imaging technologies that can visualize the microarchitecture of the male genital tract on a cellular level.

To date, several imaging technologies, such as magnetic resonance imaging,[7–9] computed tomography (CT),[8,10] positron emission tomography,[11] and diffuse optical imaging,[12] have been applied to investigate the microarchitecture of the testis and epididymis. However, all of these technologies either do not provide

satisfactory contrast or sufficient spatiotemporal resolution. Thus, spermatozoa with a head size of 4 (human) to 8 µm (most mammals) cannot be visualized. Furthermore, exposure to radiation during CT scanning might cause a transient dose-dependent negative effect on sperm concentration and quality.[13]

The new imaging techniques described in this book allow the investigation of the seminiferous tubules and the visualization of vital spermatozoa in the testis, thus opening new ways for the establishment of new diagnostic tools and the creation of new therapeutic strategies for male infertility. This might increase the outcome of assisted reproduction in males, which has high medical and emotional value.

1.3 Establishing new techniques for *in vivo* microscopy in the female genital tract

In women, infertility may be due to a variety of abdominal diseases or congenital defects. However, a large percentage of idiopathic infertilities, i.e., infertilities in which a precise cause for infertility cannot be identified by routinely used techniques, is due to impaired function of the oviduct.[14] The oviduct is not only the site of gamete transport, fertilization, and early embryonic development but also where the first early embryo-maternal communication occurs.[15,16] Only if the embryo sends specific signals to the mother and if the mother is able to respond in a precise and timely manner will successful pregnancy be established. To date, only limited techniques are available for visualization of the oviduct, which are clinically applied. Fertiloscopy is performed to obtain general information on secretory activity and appearance of the luminal surface of the uterine tube.[17] Methylene blue staining of the oviduct is used to evaluate luminal patency.[18] However, all of these techniques do not allow the visualization of the microarchitecture of the oviduct on a cellular level. Because most of the pathological alterations in the oviduct are not visible macroscopically but are only seen on a microscopic level, it is pivotal to establish new technologies that provide high resolution. In experimental studies, optical coherence tomography (OCT) has been used successfully to visualize the oviduct in mice[19] and in bovine[20] and to map quantitatively the cilia beat frequency inside the intact mouse oviduct.[21] However, up until now, only prototypes of these instruments have been available that have no license for clinical application. This is in contrast to pCLE, which is already routinely used in gastroenterology[22–24] and pulmonology[25,26] to visualize and discriminate healthy and diseased tissue during surgical procedures. Thus, this imaging technology would greatly improve precision in the diagnosis of uterine tube pathologies in the patient *in situ*. The techniques described in this book open new ways to identify the underlying cause of infertility and to create new ideas for therapeutic strategies—an essential prerequisite for increasing the success rates in assisted reproduction both in humans and animals.