COLLECTION OF GAMETES

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The first objective of IVF is the collection of oocytes and spermatozoa. Male gametes have been collected for artificial insemination or intrauterine insemination, often using a donor (IUD) over many years. Most men produce semen samples with numerous active spermatozoa. Fewer are available in cases of oligozoospermia. This had led to recent developments in the collection of epididymal spermatozoa, and even testicular spermatozoa and spermatids that are usually injected directly into ooplasm.

Collection of female gametes

Natural folliculogenesis

Following several rounds of mitotic amplification within the genital ridge of the developing fetus, oogonia enter meiosis at around the beginning of the second trimester of pregnancy and after their last round of DNA synthesis. Unlike spermatogenesis, in which stem cells persist throughout adult life and replenish the pool of developing spermatozoa, oogenesis has to supply the ovary with all germ cells required in the reproductive lifetime of a woman. This has resulted in the requirement for follicles to remain dormant but viable for up to 50 years, awaiting the signal to continue to ovulation and fertilization.

Of the many unique features of follicle development, one of the best studied and most important is meiosis, in which reductions divisions of the oocyte produce a haploid partner of the male gamete. The first stage of meiosis consists of an extended prophase I, subdivided into leptotene, zygotene, pachytene, diplotene and diakinesis. In leptotene, the chromosomes condense and become visible, although the chromatid bivalents do not appear until pachytene. Until the exclusion of the first polar body at metaphase II, the cells are diploid (2N) with a 4C DNA content. By early pachytene, the chromosomes are shorter and thicker and genetic recombination has begun, which synaptonemal complexes visible. In the fetal

human ovary, prophase I progress over a period of several weeks to halt at early diplotene, by which time the chiasmata are clearly visible. By the time of birth, virtually all oocytes are arrested in diplotene and in intimate association with a shell of somatic pregranulosa cells forming a pool of quiescent primordial follicles. The recruitment of any particular primordial follicle from the resting into the growing pool is unpredictable and occurs by unknown mechanisms. This process continues throughout life until the oocyte store is almost exhausted at menopause.

Aspiration of human oocytes from their follicles

Laparoscopic puncture
Laparoscopy was the classical method introduced by the pioneers of the IVF technique. Today the indications of follicular aspiration by the laparoscopic route are practically limited to cases in which visualization of the pelvic organs is desired simultaneously with intratubal gamete transfer (GIFT) or intratubal zygote transfer (ZIFT), i.e. cases of sterility with no apparent cause or of possible pelvic endometriosis.

Perurethral/transvesical ultrasonographic puncture

In this procedure, the bladder is filled with saline solution and, after bladder intubation with a Foley catheter, a needle is introduced through the catheter itself into the bladder. This is transfixed, and the needle penetrates the ovarian follicles. The discomfort of bladder repletion and haematuria, as well as the pain occurring after the procedure, led to a decline in the use of this route after the advent of the vaginal transducer.

Ultrasonographic puncture: transvaginal

The introduction of the vaginal route was suggested by Gleicher et al. in order to reach the ovaries high in the pelvis or fixed behind the uterus by adhesions. Since stimulated ovaries are increased in size, this would cause them to be located more frequently on the bottom of the sac, to which transvaginal puncture would have better access. However, puncture with the free hand through the fundus of the vaginal sac did not prove to be easy in all cases.

Collection of male gametes

Spermatogenesis and spermiogenesis

This brief outline describes the basic aspects of spermatogenesis and spermiogenesis. The testis fulfils a double function similar to that of the ovary, producing both sex hormones and gametes in distinct parts of the gonad. The testis consists of seminiferous tubules embedded in connective tubule tissue, which contains interstitial cells. The seminiferous tubules are responsible for the production of gametes (spermatogenesis), while interstitial cells produce hormones within the tubules, Sertoli cells form and regulate the growth and differentiation of the germ cell and developing spermatid.

Spermiogenesis

The stage includes the development of spermatids from meiosis to the detachment of the spermatozoa from the Sertoli cells. It is a truly remarkable phase, involving highly differentiated spermatozoa undergoing complex morphological, physiological and biochemical modifications. It requires approximately 22 days in humans.

This complex process of differentiation is poorly understood. It involves unique events that are tightly synchronized and integrated so that very small deviations are likely causes of infertility. These deviations are noted in mature spermatozoa and include variation in morphology, motility and function. In many animals, the young spermatids are associated in the seminiferous tubules with an older generation of spermatids formed one cycle earlier. Initially a simple cell, with major modifications in the basic cytoskeleton leads to the emergence of a highly differentiated spermatozoon. Nuclear chromatin becomes highly condensed. Cisternae of the Golgi apparatus, which are more complex than in other cells, fuse to form an acrosome. This structure then migrates to the nucleus and covers the nuclear surface as it forms the acrosome system. The inner membrane of the acrosome is closely associated with the nuclear envelope. This is one of the few secretory structures which becomes intimately associated with a nucleus.

Collection and isolation of spermatozoa for assisted conception

Sperm collection from the ejaculate

Semen is habitually collected for artificial insemination, IVF or ICSI, by masturbation into a sterile glass or plastic flask after a period of sexual abstinence ranging from 2 to 4 days. In cases of retrograde ejaculation, diabetic neuropathy, and major pelvic surgery, for example, spermatozoa must be collected in urine. In this situation, sodium bicarbonate at a concentration of 2 g is taken at bedtime and a further 2 g is taken in the morning, so adjusting urinary pH to 7. After masturbation, urine is collected into a sterile and atoxic flask and spermatozoa are separated by centrifugation.

Sperm collection from the epididymis

Several collection techniques are indicated in cases of azoospermia. These include microsurgical sperm aspiration from the epididymis (MESA), percutaneous sperm aspiration from the epididymis (PESA), biopsy and sperm extraction from the testicle (TESE), and percutaneous sperm aspiration from the testicle (TESA). The choice of method depends on various factors such as available facilities for cryopreservation and characteristics of each case, and especially on the experience of the surgeon with these techniques.

Sperm collection from the testicle

TESE has been used for many years as part of the differential diagnosis of azoospermia. The observation of abnormal spermatogenesis during this procedure would lead to a diagnosis of non-obstructive azoospermia. Testicular biopsy is an efficient method for sperm collection for ICSI. The procedure is usually ambulatory, requiring the use of a local anaesthetic. However, in some cases, it can cause great discomfort in more sensitive patients. The major acute complications are scrotal haematoma and infection. On a longterm basis, patients should be monitored for the onset of antibodies against spermatozoa, and for testicular fibrosis and calcifications followed by testicular devascularization.

Sperm collection from patients with disorders of ejaculation

The absence of ejaculation can occur for different reasons such as spinal lesions, psychosexual dysfunctions, diabetes mellitus and multiple sclerosis. Traumatic injury to the medulla resulting from car accidents, falls, and sport or violent

activities is a very frequent cause. Lesions of the spinal medulla affect a young male population (16–35 years), leading in most cases to the inability to ejaculate during intercourse. Thus, the partner will become pregnant only with the application of assisted ejaculation methods.

Conclusions

Highly novel approaches to the ovary were needed to collect mature oocytes from their follicles. Consideration of methods used to collect male gametes is opened with a brief review of human spermatogenesis and spermiogenesis. Transport through the excurrent ducts, and its control are described briefly. Sections on the collection and isolation of spermatozoa open with a brief comment on sperm collection from the ejaculate, then turn to collections from the male reproductive tract and the testis. Recent methods of aspirating epididymal spermatozoa, using percutaneous aspiration (PESA) and its value in relation to testicular extraction are described.. Brief descriptions are given of the use of spermatid as gametes, and finally the collection of spermatozoa from patients suffering disorders of ejaculation.