Bioassays of gonadotropins based on cloned receptors

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Abstract

Because of the microheterogeneities of gonadotropins, immunoreactive measurements of gonadotropins do not necessarily reflect their bioactivity. Follicle-stimulating hormone (FSH) bioassays have relied on measurement of aromatase activity in primary cultures of immature rat Sertoli cells or rat granulosa cells (GAB assay). Luteinizing hormone (LH) bioassays have relied on measurement of androgen production in primary cultures of rat interstitial testicular cells (RICT) or mouse Leydig cells. Those bioassays are cumbersome because they rely on primary culture and on indirect measurement of estradiol or testosterone by RIAs. The cloning of the cDNAs of FSH and LH receptors has allowed the establishment of cell lines expressing human receptors. The cotransfection of the recombinant gonadotropin receptor with a cAMP reporter gene allows a nonisotopic measurement of gonadotropin bioactivity. Furthermore, patient serum can be tested directly without prior extraction. We and other groups have developed a CHO cell line expressing the human FSH receptor and a luciferase reporter gene (CHO-FSHR). The CHO-FSHR assay is specific for FSH and free of serum interference up to a final concentration of 20%. The clinical sensitivity is 3 IU/l, the interCV 16%, the intraCV 8%. Studies were performed in normal women (n = 11) during the menstrual cycle using the CHO-FSHR cells. The ratio of bioactive to immunoactive FSH (B/I) equals 1.1 ± 0.04 across the follicular and early luteal phase. During the mid to late luteal phase the mean B/I rises significantly to 1.65 ± 0.07 (P < 0.001). Gonadotropin bioassays based on cloned receptors have been used to search for immunoglobulins, directed against the FSH or the LH receptors in premature ovarian failure patients. No blocking antibodies were found among the 38 women studied. A recent study of FSH bioactivity in patients with FSH secreting pituitary adenomas shows increased values of the B/I ratio. In summary, cell lines expressing the LH and the FSH human receptors are now available. Those homologous systems enable clinicians to study potential forms of mutated FSH or antibodies directed against gonadotropin receptors. Furthermore, bioassays based on cloned receptors are interesting tools to test anti-LH or anti-FSH molecules mainly in contraceptive research. Copyright © 1996 Elsevier Science Ireland Ltd.

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1. Introduction

Gonadotropins are secreted in multiple forms. Those isohormones differ by their molecular weights, charge, half-lives and biological properties. Therefore, immunoreactive follicle-stimulating hormone (FSH) or luteinizing hormone (LH) may not necessarily reflect the total biological signal as perceived by the gonadal FSH or LH receptors [1]. Several clinical cases have been reported of biologically inactive gonadotropins. The first case of a biologically inactive LH was reported in 1992 in an infertile man with a mutation on its LH β gene [2]. The first case of a biologically inactive FSH was reported in 1993 in a woman with primary amenorrhea [3]. On the other hand, an immunologically anomalous LH variant was found in healthy Finnish women. These LH variants present two mutations (1 rp en Arg6 et Ile15 en Thr15). Although they are undetectable by some immunoassays, they are biologically active [4].

Over the past 40 years, more than 15 gonadotropin bioassays have been developed. The availability of the FSH and the LH receptor cDNAs since 1990 represents a major step in the history of gonadotropin bioassays. In this chapter, we will first review the FSH and LH bioassays available before the cloning of the receptor cDNAs and point out for each bioassay its advantages.
and disadvantages. The second part will present the five different types of FSH bioassays and the two different types of LH bioassays based on cloned receptors, described so far. We will then present the results already obtained with those bioassays. Finally, we will criticize those ‘new generation’ bioassays.

2. Bioassays available before the cloning of gonadotropin receptors

2.1. FSH bioassays

Five main types of FSH bioassays are currently being used: the in vivo Steelman and Pohley bioassay, the radioligand receptor assays (RRA), the in vitro Sertoli cell bioassay, the in vitro Granulosa cell bioassay and the inhibin immunoassay.

2.1.1. The FSH in vivo bioassay or Steelman and Pohley bioassay

One must remember that numerous attempts to quantitatively assay FSH have started since 1937 by measuring the increase in ovarian weight in immature female rats, long before the arrival of FSH immunoassays in 1965. Steelman and Pohley first described in 1953 an FSH bioassay in intact immature female rats [5]. This bioassay is based on the fact that 22-day-old female rats pretreated with hCG are sensitive to exogenous FSH and that the relationship between administered FSH and ovarian weight is linear. The FSH injection is performed once a day for 3 days. The rat autopsy is performed at 72 h. In the original report, the average ovarian weight was 12.9, 14.8, 19.6 mg with respectively 0.1, 0.2 and 0.3 mg of a swine FSH preparation. This method is therefore cumbersome and hard to reproduce. Furthermore, its sensitivity is low and this bioassay is highly sensitive to serum interference. Serum interference is a non-specific effect due to unknown molecules contained in the serum inducing stimulation or inhibition of FSH bioactivity. Therefore, it cannot be used for measuring FSH bioactivity in serum. One of the main advantage of this in vivo bioassay is that it takes into account the clearance of the hormone. It is still being used to measure FSH in highly purified pituitary and urinary extracts.

2.1.2. The FSH RRA

L.E. Reichert et al. described in 1973 a RRA for human FSH [6]. Mature rats of the Sprague Dawley strain are used to obtain a testes tubule receptor preparation. The RRA relies on the binding competition on the membrane preparation between iodinated FSH and the FSH to measure. This bioassay although sensitive is sensitive to serum interference. Different types of RRA have been developed since then using bovine calf testis membranes [7,8].

Serum samples from normal menstruating women were tested directly without extraction or dialysis using the calf testis membrane RRA [8]. The RRA/RIA ratio for the early follicular phase was 0.61 ± 0.27 (mean ± S.E.M.) for the late follicular phase 0.65 ± 0.3, for the mid-cycle 0.48 ± 0.15 and for the luteal phase 0.7 ± 0.48. RRAs have been used mainly to characterize FSH preparations and more recently recombinant human FSH [9]. They also have been used in premature ovarian failure patients to look for FSH binding inhibitors in those patient’s serum [10].

However, this bioassay is not devoid of serum interference. Furthermore, the binding of FSH to its receptor as measured in the RRA does not necessarily correlate with its bioactivity.

2.1.3. The Sertoli cell bioassay

2.1.3.1. Principle of the Sertoli cell bioassay. In 1979, Van Damme et al. developed an in vitro bioassay based on the measurement of estrogen production in cultured Sertoli cells from 10-day-old rats [11]. This assay is based on the observation by Dorrington and Armstrong in 1975 that a dose response relationship exists between the amount of estradiol produced when FSH but not LH is added to cultured Sertoli cells obtained from immature 10 to 20-day-old rats. Exogenous testosterone is converted to 17β estradiol in the presence of FSH.

Other end points than estradiol production have been used in other FSH bioassays based on Sertoli cell culture. In 1975, Dorrington and Armstrong proposed instead of measuring estradiol by RIA, a measurement of aromatase activity that does not rely on an RIA, by measuring $3H_2O$ release from $1β^3$-H testosterone [12]. The Sertoli cell bioassay previously described, has been improved by V. Padmanabhan et al. [13] in 1987. The culture conditions were optimized within a serum-free medium, and the sensitivity increased. 7–10 day-old Sprague-Dawley rats were used to obtain Sertoli cells. After an initial incubation of the cells for 72 h, the serum-free culture medium is removed. FSH standards or unknown samples, MIX and 19 OH androstenedione are incubated for 24 h. The medium is then aspirated and estradiol measured by RIA. The mean intra- and interassay coefficients of variation were 9 and 11%. The sensitivity was 0.01 mg/ml of NIAMDD hFSH-2. This bioassay can be used to measure FSH in serum without using an extraction.

2.1.3.2. Different uses of the Sertoli cell bioassay. (a) Human menstrual cycle. The FSH bioactive/imunonactive (B/I) ratio was the lowest (1.6 ± 0.4) during the luteal phase of the menstrual cycle and increased (P < 0.001) by a factor of approximately 4 at the time of the midcycle gonadotropin surge (6.2 ± 1.4). B/I
ratio are higher in menstruating females than in males and increase with age.

(b) Fetal blood samples. In a study of 114 normal human fetuses, the B-FSH concentrations are higher than the I-FSH levels. Therefore, an FSH-like material could be capable of inducing aromatase activity in rat Sertoli cells [14].

(c) Puberty. FSH bioactivity was measured in 28 healthy boys between Tanner stage I and Tanner stage V. The B-FSH was relatively constant over the pubertal stages [15].

(d) Recombinant human FSH hormone. Recombinant FSH, urinary and pituitary FSH displayed comparable activities using the Sertoli cell bioassay when adjusted for the immunoactive level [9].

2.1.4. The granulosa cell aromatase bioassay (GAB)

2.1.4.1. Principle of the GAB assay. The GAB is based on the measurement of estrogen production by primary cultures of intact Sprague–Dawley rat granulosa cells [16,17]. Rats are implanted with Silastic capsules containing DES to stimulate granulosa cell proliferation. Animals are killed 4 days after implantation and their ovaries dissected for granulosa cell culture. Cells are grown in the presence of a phosphodiesterase inhibitor MIX (0.125 mM), insulin (1 µg/ml), hCG (30 ng/ml), DES (10⁻⁷ M) and androstenedione as the aromatase substrate (10⁻⁶ M). At the end of the 3 day culture, medium estradiol content is measured by RadioimmunoAssay (RIA). The inter and intra-assay coefficient of variation of the GAB bioassay are 18 and 13%, respectively. The assay is not species-specific: rat, human, ovine FSH can be measured in the bioassay.

Other end points than estradiol production have been used in cultured granulosa cells. In 1978, Beers and Strickland proposed a bioassay based on the stimulation of plasminogen activator activity secreted by cultured granulosa cells. However, this bioassay was sensitive to both FSH and LH. Others have measured ³H glucosamine incorporation into proteoglycans, progesterone secretion from granulosa cells obtained from immature hypophysectomized diethylstilbestrol-primed rats.

One of the drawbacks of this bioassay is that serum needs to be pretreated with polyethylene glycol (PEG). This extraction procedure removes unknown serum factors interfering with the bioassay. Therefore PEG may fail to assess accurately the FSH bioactivity by removing low molecular weight inhibitors.

The GAB assay was further improved [18]. The addition of IGFl (10 µg/l), transferrin (50 mg/l) and progesterone (100 nM) increased the assay sensitivity. The reported sensitivity of the assay is 3–4.5 IU/l. The interassay coefficient of variation was 13 ± 3%. The GAB assay was also modified by Matikainen et al. [19].

2.1.4.2. Uses of the GAB assay

(a) Human menstrual cycle. During the human menstrual cycle, the FSH B/I ratio throughout the menstrual cycle ranged from 1.4–3.4 with a mean of 2.5 [17]. The ratios for the follicular phase, late follicular phase, preovulatory surge and luteal phase were 2.7, 2.3, 1.4 and 2.6 respectively. The correlation coefficient of the serum FSH values obtained by bioassay and RIA was 0.91. Using the ‘modified’ granulosa cell bioassay to study women during normal cycle, B FSH was close to I FSH, r = 0.79, P < 0.001. B-FSH was elevated during the early follicular phase and during the mid-cycle surge, lowest during the luteal phase [18].

(b) Urinary FSH during the human cycle. The first morning FSH bioactivity reflects serum bio-FSH concentration. However, the concentration of urinary bio-FSH was 50–100-fold higher than the serum level [21].

(c) Postmenopausal women treated with GnRH agonist and antagonist. The antagonist treatment (Nal–Glu) of postmenopausal women decreased the FSH B/I ratio (6.4 ± 1.7 to 4.5 ± 1.0 (P < 0.05)) and the agonist treatment (D-Trp⁵-GnRH) increased the FSH B/I ratio [19]. Interestingly, no changes in the B/I ratio of LH were found after either treatment.

(d) Women with cycle abnormalities, with or without polycystic ovarian disease. No difference was found in the B/I ratio between normal women, PCOD and women with cycle abnormalities non PCOD [22].

(e) Men treated with GnRH agonists and antagonists. FSH bioactivity does not decrease significantly during 6 months treatment with a GnRH agonist analogue in patients with prostatic carcinoma [23]. After the administration of a GnRH antagonist, the FSH level is suppressed to a greater extent than are immunoreactive levels [24].

(f) Recombinant FSH hormone and partially deglycosylated variants. The in vitro bioactivity of FSH variants produced either by CHO mutant cells deficient in the glycosylation enzyme N-acetylglucosamine transferase-I or CHO mutant cells defective in sialic transport into the Golgi were studied in the GAB assay [25]. Both FSH variants are as active as FSH secreted by the wild type CHO cells and purified pituitary FSH. However, those variants exhibited minimal in vivo bioactivity.

2.1.5. The inhibin immunoassay

Inhibin is a peptide consisting of an α-subunit linked by two highly homologous β-subunits to form either inhibin A (α-βA) or inhibin B (α-βB). It was originally isolated from follicular fluid [26]. Although the inhibin level contributes to the modulation of FSH secretion by pituitary gonadotropes, it is known that FSH stimulates inhibin production by granulosa cells or Sertoli cells in vitro. However, the use of inhibin as an FSH bioassay is controversial. One of the main reason is the
difficulty to obtain a sensitive immunoassay which does not cross react with potentially inactive precursor forms of inhibin. The second main difficulty is to distinguish the physiological active forms of inhibin from the inactive forms. Recently, N. Groome has reported using a specific inhibin B assay that inhibin B appears to be the predominant form of inhibin in the preovulatory follicle [27]. The timing of the early rise in the plasma inhibin B concentration suggests that it is secreted by antral follicles in response to FSH.

2.2. LH bioassays

2.2.1. LH in vivo bioassay

In 1941, Greep first described the use of the anterior lobe of the prostate gland or the testes weight in the assay of metakentrin later called LH [28].

2.2.2. LH radioligand receptor assay

The LH radioligand receptor assay have relied primarily upon rat testis homogenate [29] or rat corpora lutea [30], tissues rich in LH/CG receptor. Another type of RRA has been developed by Whitcomb et al. using the cell line MA-10 [31]. This stable line of murine Leydig tumor cells provided from Dr Mario Ascoli expresses the LH/CG receptors.

2.2.3. The rat interstitial testosterone production (RITC) bioassay

M. Dufau et al. described a highly sensitive in vitro bioassay for Luteinizing Hormone and Chorionic Gonadotropin relying on testosterone production by dispersed rat Leydig cells [32]. Testes are removed from adult Sprague–Dawley rats. The interstitial cells are collagenase-dispersed in the presence of phosphodiesterase inhibitors. The sensitivity of the assay is 20 µIU (2 picograms) of hCG.

2.2.4. The mouse Leydig cell bioassay

Van Damme described an LH bioassay where the end point is testosterone production by mouse Leydig cells [33].

In summary, one of the main problems concerning the FSH or LH bioassays previously described, except for the MA-10 cell line bioassay is their need for primary cell culture. Furthermore, those primary cultures mainly rely on murine cells which represent a nonhomologous system when serum or purified immunoglobulins from patients are being tested. In addition, those systems need an indirect measurement of estradiol by RIA, their sensitivity may be hard to achieve for patient’s samples and some are subject to serum interference. Indeed, serum proteins exert an inhibitory effect in those in vitro bioassays. The response can therefore be modified by nonspecific serum effects or by non-LH and non-FSH factors such as epidermal growth factor, IL-1, insulin-like growth factor-1. Furthermore, there are discrepancies between the results from different bioassays. For instance, the FSH-bioactive levels differ during the human menstrual cycle, between the Sertoli cell bioassay and the granulosa cell bioassay.

3. Bioassays based on cloned receptors

3.1. FSH bioassays

The rat FSH receptor gene was cloned in 1990 [34]. Several groups have since then cloned the human FSH receptor cDNA [35–37]. The FSH receptor protein belongs to the G-protein coupled receptor family. When FSH binds to its membrane receptor, adenyl cyclase activity increases through interaction with membrane associated G-proteins. The cAMP increase can be quantified directly by cAMP RIAs or through the use of reporter genes containing cAMP Responsive Elements (CREs) in their promoter. Five different FSH bioassays based on cloned receptors have been described in the literature so far.

Gudermann et al. in E. Nieschlag’s group has described an in vitro bioassay based on L-cells transfected with the recombinant rat FSH receptor [38]. Those cells express approximately 10,000 receptor sites per cell. The end point in this bioassay is the cAMP production measured by RIA. Cells are grown in the presence of 10% fetal calf serum. To avoid interfering serum factors, a 10 min heat treatment at 56°C is applied to all serum samples. The sensitivity of the assay is 0.3 IU/l which is equivalent to 3 IU/l serum FSH in terms of WHO FSH standard 78/549. The intraassay coefficient of variation is 5.2%, the interassay coefficient of variation is 16.2%.

Tilly et al. in Hsueh’s group used a human fetal kidney cell line (293) transfected with the human FSH receptor and a luciferase reporter gene driven by a cAMP-responsive region of the rat tPA gene promoter [36].

Chappel et al. transfected a mouse adrenal cell line with the human FSH receptor (Y1-hFSHR). These cells exhibit a dose-dependent increase in progesterone when exposed to hFSH [37].

Tano et al. used Chinese hamster ovary cells transfected with the recombinant human FSH receptor [39]. Intracellular cAMP production is measured by RIA. The incubation of the bioassay lasts 60 min at 37°C. 10% FSH free serum are added with the standards to compensate the serum interference. The sensitivity is 6.2 IU/l of FSH-I3. The intra-assay and interassay coefficients of variation are 7.3 ± 0.5 and 10.3 ± 0.5%, respectively.
The CHO-luciferase FSH bioassay relies on CHO cells cotransfected with the human FSH receptor cDNA and a luciferase reporter gene [40]. The reporter gene is made of the human glycoprotein hormone x (GPHx) gene which contains one of the most studied cyclic AMP responsive promoters linked to the luciferase gene. The cAMP response is mediated by interactions between cyclic AMP responsive element binding protein (CREB) and tandem copies of a CRE. The number of receptors per cell in this cell line was estimated by a Scatchard analysis to be 16,000 ± 5,000. During the bioassay, cells are incubated for 5 h with the standards or the serum to be tested. After the cell lysis, luciferase activity is measured directly by a luminometer. This bioassay is specific for FSH as LH, rTSH, hCG, human GH or prolactine do not generate light unit production. The limit of detection of this bioassay is 0.1 IU/l of hMG and the serum sensitivity equals 3 IU/l. The intra and inter coefficients of variation are 8 and 16% respectively.

3.2. LH bioassays

As the LH receptor cDNA has been cloned [41,42] bioassays based on cloned receptors have been developed.

Jia et al. in Hsueh’s group described in 1993 an LH/CaCG bioassay based on human fetal kidney 293 cells permanently transfected with the human LH receptor cDNA and a luciferase reporter gene driven by a cAMP dependent promoter [43]. A DNA fragment of the thymidine kinase (tk) promoter region and the mouse inhibit x gene containing a cAMP-responsive element (CRE) were subcloned into the luciferase expression vector containing the entire firefly luciferase coding region. Cells are treated for 20 h. The minimal effective dose is 0.3 ng/ml of LH. The inter and intra assay errors expressed as coefficients of variation of pooled serum were 13 and 18% respectively. The sensitivity of this bioassay as described in 1993 was less than the rodent Leydig cell bioassay.

Jameson’s group has set up an LH bioassay using the same luciferase reporter gene as in the CHO-FSH bioassay previously described.

The list of FSH and LH bioassays described in this chapter is of course not exhaustive.

4. Clinical use of bioassays based on cloned receptors

4.1. The human menstrual cycle and postmenopausal women

We have used the CHO-FSHHR cell line to characterize the FSH bioactivity over the human menstrual cycle [44], in 12 normal women as shown in Fig. 1. FSH bioactivity did not change substantially across the follicular phase. FSH bioactivity increased on the day of the midcycle preovulatory gonadotropin surge in parallel with FSH immunoreactivity. A second and more sustained elevation of FSH bioactivity began during the mid to late luteal phase, the FSH B/I ratio was significantly higher than the B/I ratio across the rest of the cycle (1.65 ± 0.07 versus 1.1 ± 0.04, P < 0.001).

11 postmenopausal women were also studied. FSH bioactivity ranged from 65 to 200 IU/l. The mean B/I ratio of 0.93 ± 0.11 did not appear to change over a wide range of FSH immunoactivity.

4.2. Premature ovarian failure (POF)

POF is defined by a menopause occurring in a woman between the age of 18 and 40. The etiology of this syndrome remains unclear although an autoimmune origin has been suggested by many authors. Several groups have looked in POF patient’s serum for antibodies directed against the FSH or the LH receptor. Chiauzzi et al. found using an FSH RRA an IgG interfering with the binding of FSH to its receptor in two patients with premature ovarian failure and myasthenia gravis [45]. The FSH and LH bioassays based on cloned receptors have been recently used to look for antibodies directed against the LH and the FSH receptor.

Anasti et al. have used the Y1-hFSHR cells to look for anti-FSH receptor antibodies and hLHR-293 cells to look for anti-LH receptor antibodies in 38 premature ovarian failure women [46]. In Y1-hFSHR cells, the end point was the measurement of progesterone production. In hLHR-293, the end point was the measurement of cAMP. Each serum sample was fractionated by recombinant protein-G affinity chromatography. The IgG purified were tested in both FSH and LH bioassays. No blocking antibodies to FSH or LH receptors were identified in any of the POF patients.

Tano et al. using the cell line have tested 12 women aged 27 to 39 years with POF and nine postmenopausal women [39]. The immunoglobulin (IgG) fractions of one patient showed a stimulatory effect on cAMP production compared with that from postmenopausal women. The IgG from another patient showed an inhibitory effect on cAMP production. Somehow, the authors do not give the FSH bioactive values of all the POF women and the amount of POF patients tested for IgG fractions are not given in the paper. Furthermore, the effect reported is only on basal cAMP production and not on the FSH stimulation.
5. Advantages and inconveniences of bioassays based on cloned receptors

5.1. Advantages of bioassays based on cloned receptors

- A permanent cell line is used in bioassays based on cloned receptor. They avoid primary culture of granulosa, Sertoli or Leydig cells and no live animals are used.

- The FSH or LH bioactivity is measured directly by a luminometer when a luciferase reporter gene is transfected in the cell line. No radioactivity is needed.

- Those systems are homologous for the human species as the cell line expresses the human FSH receptor (except for the FSH bioassay).

- These bioassays are subject to fewer serum interference than ‘usual’ bioassays.

- These bioassays can be used to measure FSH and LH in species where immunoassays are difficult to obtain. For example, the LH/CG luminescence bioassay can be used to measure monkey CG.

5.2. Inconveniences of bioassays based on cloned receptors

The biological activity of FSH measured by the bioassays based on cloned receptors only measures the FSH bioactivity occurring through the cAMP pathway. Although cAMP is the primary second messenger for the action of FSH, it has been shown that FSH increases the intracellular calcium level in rat Sertoli cells. Therefore, the hypothesis that the FSH receptor could be a calcium channel was suspected. However patch clamp studies using 293 cells have given evidence that the FSH receptor itself is not a calcium channel [47].

There is still a debate about the human FSH and CG/LH receptor specificity. According to Tilly et al., the hFSH receptor interacts preferentially with human and rat FSH. hFSH and rFSH preparations were effective in binding to the recombinant hFSH receptor in 293 cells whereas only minimal interaction of oFSH, pFSH or eCG with hFSH receptors was observed. Therefore a bioassay expressing the human receptor could only be used to measure higher species. On the contrary, FSH from rat, human and ovine origin as well as eCG effectively competed with radiolabeled FSH for binding sites in rat testicular homogenates and in RRA using recombinant human receptor [48]. Furthermore, ovine, rat or bovine FSH enhance the relative light unit production in the FSH CHO-luciferase bioassay.

Similarly, the same group has shown that the human LH receptor binds human but not equine, rat and ovine LH and CG indicating the species specificity of the receptor [49].
Some of the bioassays based on cloned receptors described are not devoid of serum interferences although they are much less sensitive to serum inhibition. The potential mechanisms for those interferences are still debated. They could involve a cytotoxic effect of serum [38] or an effect via the FSH receptor (Rommerts personal communication).

The FSH bioactivity is tested only after binding of FSH to the full length receptor and not to the potential other FSH receptor isoforms. According to Albanese et al. using a human ovarian cDNA library, three FSH receptor isoforms different from the full length receptor were found. One of these isoforms was deleted from an arginine codon representing the first amino acid residue in exon 4. It is also deleted from sequences corresponding to putative exon 2 and exon 6. Another clone contains a 50 bp insertion corresponding to the junction between exons 8 and 9 plus an in frame deletion of exon 6. Finally, the third clone contains an intron deletion of exon 6. Those isoforms probably result from incomplete or aberrant RNA splicing. The meaning of these isoforms in physiology is however still unknown.

Those bioassays based on cloned receptors have a sensitivity close to the 'classic bioassays'. Those systems based on molecular biology have not enabled us so far to improve the clinical sensitivity. For LH bioassy, the sensitivity up to now is even less than that of the rodent Leydig cell bioassay. Improvement of sensitivity might occur by using different promoters linked to the reporter gene.

6. Conclusion

In this chapter, we have reviewed the different types of LH and FSH bioassays: the 'classic bioassays' and the 'new generation' bioassays based on cloned receptors. However, this review could not be exhaustive in the description of every bioassay or in their potential use, especially to characterize isoforms or to identify synthetic peptide bioactivities. We have presented the different types of bioassays based on cloned receptors described so far with their advantages and disadvantages. Although their sensitivity is roughly similar to 'classic bioassays', they are much easier to perform because they rely on stable cell lines. Furthermore, most of them are homologous when studying human serum or human purified immunoglobulins. Finally, they are much less sensitive to serum interferences.

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