Complete Selective Absence of Protamine P2 in Humans*

(Received for publication, December 30, 1992, and in revised form, February 11, 1993)

Lluisa de Yebra‡, José Luis Ballescà§, Juan Antonio Vaarell§, Lluis Bassas¶, and Rafael Oliva†

From the ‡Molecular Genetics Research Group, Faculty of Medicine, University of Barcelona, 08028 Barcelona, the §Obstetrics and Gynecology Unit, Hospital Clinic i Provincial, 08006 Barcelona, and the ¶Puigvert Foundation, Hospital de la Santa Creu i Sant Pau, 08025 Barcelona, Spain

Male sterility due to abnormal sperm morphology or motion has been widely reported, although relatively little has been published on the sperm nuclear protein abnormalities. We report the first cases worldwide of infertile patients having a complete selective absence of protamine P2 in the sperm nucleus. This provides a selective phenotype that will aid understanding of the mechanisms of synthesis, processing, or function of the P2 protamines. In addition, it is of marked immediate relevance to medicine as it allows the diagnosis of this type of human male sterility and the opportunity to understand the basis of this defect.

The sperm nucleus is of special interest to researchers not only because it delivers the haploid genetic complement, but also because of its marked compositional and morphological changes as compared to the rest of the nucleus in the organism. The nucleohistone structure present in spermocytes is disassembled during spermiogenesis in many species and replaced by a complex formed by the DNA condensed by protamines (for reviews see Mezquita (1985), Focia (1986), Balhorn (1989), Hecht (1989), Kasinsky (1989), and Oliva and Dixon (1991)). In humans, protamines are the most abundant nuclear proteins in the sperm nucleus packaging the male genome. There are two types of protamines: P1, which is present in all mammalian species studied so far and is rich in arginine and cysteine (Tobita et al., 1983; Sautiere et al., 1984; McKay et al., 1985; Lee et al., 1987; Domenjoud et al., 1990; Queralt and Oliva, 1991; Winkfein et al., 1991; Balhorn et al., 1992; Adroer et al., 1992; Retief et al., 1993), and the P2 family, composed of the P2, P3, and P4 components, which differ only by an amino-terminal extension of 1-4 residues (McKay et al., 1986; Arghis et al., 1991). Unlike P1 protamines, P2 protamines are only present in humans and a few other mammals (mouse, hamster, and stallion among others) and are in addition histidine-rich (McKay et al., 1986; Gussie et al., 1986; Bower et al., 1987; Lescoat et al., 1988; Sautiere et al., 1988; Bellvé et al., 1988; Domenjoud et al., 1988, 1991; Lee et al., 1991; Arghis et al., 1991; Oliva and Dixon, 1991; Chauvière et al., 1992; Pirhonen et al., 1990). The functional and evolutionary differences between P1 and P2 protamines are not completely understood at present. In addition to having been found in all mammals studied, P1-type protamines have also been found in birds (Oliva and Dixon, 1989; Oliva et al., 1989), suggesting a more universal role of P1 protamines in the sperm nucleus than the P2 protamines, which are found only in some mammals. It has been demonstrated that bull, boar, and rat (which lack P2 in the sperm nucleus) still contain the P2 gene in their respective genomes (Tanhauser and Hecht, 1989; Bunick et al., 1990; Maier et al., 1990). The lack of P2 in boar and bull has been attributed to mutations in the genes and respective primary sequence of the protein, which could weaken the affinity to DNA (Maier et al., 1990). In the case of the rat, evidence for the presence of P2 precursor in the mature rat sperm has been presented (Stanker et al., 1992; Krenling et al., 1992). In humans, slight alterations in the P1/P2 ratio have been reported in different studies (Chevallier et al., 1987; Balhorn et al., 1988; Bach et al., 1990; Blanchard et al., 1990; Belokopytova et al., 1993) in a few patients (2-17; altered P1/P2 ratio = 1.58 ± 0.24, range 1.2-1.94; normal P1/P2 ratio = 0.98 ± 0.12, range 0.79-1.27) (Balhorn et al., 1988). In this study we have analyzed the sperm samples from 116 random unselected patients from infertile couples. Because of the large number of samples analyzed, we have been able to detect new sperm nuclear compositional differences and trends among samples. One of the most remarkable findings has been the detection of a complete selective absence of protamine P2 in the sperm nuclei of 4 patients.

EXPERIMENTAL PROCEDURES

Unselected human sperm samples (ejaculates) from infertile couples attending the first visit to the infertility clinic were collected and kept frozen until processed. Approximately 14 × 10⁶ sperm cells from each sample were thawed and spun in 1.5 ml tubes in a microcentrifuge at 12,000 rpm at 4 °C for 5 min, the supernatant discarded, and the sediment washed twice (resuspending the cells by vortexing 10 s and centrifuging the cell suspension 5 min each time) with 200 μl of freshly prepared 1 mM phenylmethylsulfonyl fluoride (Sigma) in H₂O (to lyse the cells by hypotonic shock). The sediment was then processed and analyzed by polyacrylamide gel electrophoresis as described (Yebra and Oliva, 1993). Polyacrylamide gel electrophoresis was performed on 23-cm gels containing 0.9 M acetic acid, 2.5 M urea, 15% acrylamide, 0.09% bisacrylamide, 0.12% ammonium perfluorooctane, 0.4% TEMED, 2% The gels were then dried between two sheets of cellophane film and the intensity of the bands quantified by scanning each lane (Hoeffer Scientific linear scanner) and subsequently measured.

1 The abbreviation used is: TEMED, N,N,N',N'-tetramethylethylenediamine.
uring the area of each peak with a digitizing tablet (Kontron). The ratio P1/P2 was then calculated, and the samples were ordered and plotted according to decreasing P1/P2 ratios (Fig. 2A). The ratio to total nuclear protein of the intermediate proteins (Fig. 2B), proteins with a migration similar to histones (Fig. 2C), and total protamines (Fig. 2D) were also calculated and expressed in function of samples ordered in decreasing P1/P2 ratios. The ratios P1/P2 for each sample was also plotted as a function of the different parameters studied in order to further demonstrate correlations among parameters (Fig. 3).

RESULTS

Analysis of the sperm nuclear proteins from 116 different individuals revealed that 22.4% of these had a normal P1/P2 ratio (1.10 ± 0.08, range 0.92–1.27) and 74.1% had an altered P1/P2 ratio (3.00 ± 2.84, range 1.28–15.52). Unexpectedly, four (3.4%) samples had a complete lack of P2 protamine in their sperm nucleus, whereas their protamine P1 levels were conserved (Fig. 1). Sperm count corresponding to the samples with a complete selective absence of P2 was 10.6 ± 7.1 × 10^6 sperm cells/ml, the ejaculate volume was 3.8 ± 1.4 ml, abnormal sperm cells were 63.5 ± 11.4%, and astenozoospermia was present (progressive motility was 3.3%, range 0–13.3%; difficult progressive motility 18.4 ± 9.1%; and non-progressive motility was 0.8%, range 0–3.3%). Because of the large number of samples analyzed, many new trends have also emerged (Figs. 2 and 3). 1) Samples with a higher P1/P2 ratio (lower P2) also contained a higher proportion of intermediate proteins (see Figs. 2B and 3B). 2) The proteins with a migration similar to that of histone standards covered a wide range of levels (Figs. 2C and 3C). A large proportion of the samples with an altered P1/P2 ratio also had an increased level of proteins with a mobility similar to histones (Fig. 3C). 3) Samples with an increased P1/P2 ratio (lower P2) also had decreased total protamine levels (Figs. 2D and 3A). 4) Samples with a low sperm count (below 25 million/ml) were also more likely to have an altered P1/P2 ratio (Fig. 3D).

With the above data, it is now possible to propose a more detailed classification of the human sperm nuclear protein ratios as follows: 1) complete selective absence of P2 (3.4%),

![Fig. 1. Sperm nuclear proteins analyzed by polyacrylamide gel electrophoresis (Bach et al., 1990, Yebra and Oliva, 1993). Lane 1, standard of chicken erythrocyte histones; lane 2, infertile patient with a complete absence of protamine P2; lanes 3 and 4, infertile patients with altered and normal P1/P2 ratios, respectively. INTERMEDIATE PROTEINS refers to human transition proteins 1 and 2 as well as P2 family precursors (HPS1 and HPS2) at early stages of processing among others (Gusse et al., 1986; Sautière et al., 1988; Luerssen et al., 1990; Schütter et al., 1992). P1, protamine P1. P2 family, protamines P2, P3, and P4, which have been sequenced and differ only by an amino acid extension of 1–4 residues (Arkhis et al., 1991).](image1)

![Fig. 2. A, ratio P1/P2 versus sample number ordered according to the decreasing P1/P2 ratio. The order of the samples in the subsequent plots (B–E) is the same as in this graph. B, ratio of intermediate proteins (HTP1, HTP2, HPS1, HPS2, and others) to total nuclear proteins. C, ratio of proteins with a migration similar to that of a histone standard (present in another lane) to total nuclear proteins. D, ratio of total protamine (P1 + P2 family) to total nuclear protein. E, ratio P2/P3.](image2)
Protamine P2 Absence in Humans

Fig. 3. Ratio P1/P2 plotted as a function of different parameters measured. A, ratio P1/P2 versus ratio of total protamine (P1 + P2 family) to total nuclear protein (prot/tot). B, ratio P1/P2 versus ratio of intermediate proteins HTP1, HTP2, HPS1, HPS2, and others to total nuclear proteins (int/tot). C, relation between P1/P2 versus the relative level of proteins with a mobility similar to that of a histone standard. D, ratio P1/P2 versus the sperm count of the different samples.

2) altered P1/P2 ratio with increased levels of intermediate proteins above 0.3 with respect to total nuclear protein (61.2%), 3) altered P1/P2 ratio with levels of intermediate proteins below 0.3 respect to total nuclear protein (12.9%), 4) normal P1/P2 ratio but increased levels of intermediate proteins over 0.3 with respect to total nuclear protein (11.2%), 5) normal P1/P2 ratio with levels of intermediate proteins below 0.3 with respect to total nuclear protein (11.2%). In 1 of these 4 patients, an independent semen sample obtained 6 months later also reproducibly demonstrated the complete selective absence of P2 family proteins. This indicates that this phenotype was present in this patient at least during this period of time.

DISCUSSION

In this paper we have analyzed the nuclear proteins of 125 sperm samples from different human individuals and detected the first examples worldwide of a complete selective absence of protamine P2 in the human sperm nucleus as well as many different new trends in the ratio of sperm nuclear proteins. This is the most extensive study (116 different patients plus 9 controls) performed so far analyzing the human sperm nuclear proteins. The absence of identification of this novel type of alteration by previous authors most likely reflects the small number of infertile patients previously studied by them (between 2 and 17 at most), although epidemiological or ethnical differences could also be present. The much larger proportion of patients having only a decrease in the levels of P2 compared with the small number of patients having a complete selective absence of P2 suggests that the most frequent type of alteration could be a heterozygous form of a genetic defect causing the complete selective absence of protamine P2 when in a homozygous state. Several families with multiple infertile males have been described (Cantu et al., 1981; Chaganti et al., 1980; Florke-Gerloff et al., 1984). The average number of years of sterility history in the selective P2 absence patients is 6.4 ± 0.3. None of these patients has ever been a father.

The detection of this selective absence of P2 protamine in the sperm nucleus offers a unique opportunity to determine the mechanisms leading to this type of sterility. For instance, the complete absence could arise either from a lack of expression of the P2 gene or from anomalous processing of the P2 precursor and subsequent transport into the nucleus and binding to the DNA. In the boar, it has been shown that the lack of P2 in the sperm nucleus is due to mutations within the P2 gene (Maier et al., 1990). We have sequenced the protamine genes from these patients in search for mutations. Preliminary results indicate that no mutations are present at the coding region level or in the promoter up to -200 base pairs from the transcriptional start site in any of these four patients. In order to narrow down the search for the primary defect, we have also initiated the study of the expression of the protamine genes in testicular biopsy samples in search for altered expression or processing of the P2 precursor. It is not clear whether the lack of P2 in this patients is the primary
defect leading to sterility or whether it merely represents an indirect factor contributing to sterility due to a different primary cause. Many factors that impair spermatogenesis (toxic agents, deficiencies, infections, immunological changes, and hormonal changes) could lead to oligosperma and/or decreased P2 levels (Aiman et al., 1979; Snyder, 1990; Ueda et al., 1991; Fraga et al., 1991; Jégou et al., 1991; Scaglia et al., 1991; Pavlou et al., 1991; Schlegel et al., 1991; van Beek and Meistrich, 1992). It should also be considered a possibility that several independent causes (for example different diseases) affecting spermatogenesis could all lead through independent mechanisms to a common alteration in the levels of P2 proteins, ranging from slight changes in the P1/P2 ratio to the complete selective absence of P2.

Decreased levels of P2 in the nucleus could originate in the final stages of spermiogenesis before the synthesis of P2 has been completed or processed. In the mouse it has been shown that the initiation of the synthesis of the “tyrosine variants of protamine” (P1) must be separated by at least 1 day by the synthesis of the “histidine variants of protamine” (P2) (Balhorn et al., 1984). An uncoupling in the generation of mature forms of P1 and P2 protamines could be originated by the fact that P2s are synthesized as precursors that must be processed, whereas P1s are not. A general failure of the processing of proteins (including P2s) or of the nucleohistone to nucleoprotamine replacement mechanisms (Oliva et al., 1987, 1990; Oliva and Dixon, 1991) at this level, which could be caused by different defects or agents (see above), should result in a relative decrease or the absence of P2 protamines, in the gametic cells found in the semen. The presence of increased levels of intermediate proteins in samples with lower P1/P2 ratios (Figs. 2B and 3B) may indicate incomplete processing of P2 proteins.

In addition to the immediate relevance to medicine, the findings reported here also represent a unique opportunity to understand many basic aspects of the expression, control, and function of the protamines. The fact that all of the P2 family components have disappeared could support the idea that all P2, P3, and P4 are derived from a single gene (Arkhis et al., 1991). Balhorn et al. (1988) have reported that the levels of P2 decrease in infertile patients, while the levels of P3 are indistinguishable from that of normal samples. Our results indicate that the ratio P2/P3 (control = 2.48 ± 0.54; infertile group = 2.73 ± 0.68; Fig. 2E) is comparable to that detected by Belokopytova et al. (1988) (control group = 3.17 ± 0.68; infertile group = 3.23 ± 0.25), although we have not detected a significant correlation between altered P1/P2-family ratios and the ratio P2/P3 after analyzing samples from 116 different patients (Fig. 2E). Belokopytova et al. (1993) suggest that in normal sperm P2s bind more strongly to DNA than P1s but that the affinity of P2s to DNA in infertile patients has been reduced. Since we have not detected any mutations at the coding region level of the protamine P2 genes in any of these four patients lacking P2, the hypothesis of mutations causing decreased affinity of mutated P2s to DNA can be discarded in these cases. Despite the extremely positively charged nature of the protamines and the obvious participation in condensing the DNA in the sperm nucleus, the basic function of the protamines remains unknown at present (Oliva and Dixon, 1991). The basic hypotheses are: 1) condensation and streaming of the sperm nucleus, 2) protection of the genetic message delivered by the spermatozoa, and 3) generation of a compacted/blank state in the male genome (Oliva and Dixon, 1991).

The complete selective absence of P2 in these patients should lead to a loss of the structural, biochemical, and functional aspects normally provided by this protamine. In this sense we have initiated the study of the sperm morphological nuclear changes of samples having a complete selective absence of protamine P2 as compared to normal sperm containing the full complement of P1 and P2 protamines. Preliminary results indicate a marked decondensation of the P2 lacking nuclei as compared to the samples having a full complement of P1 and P2 protamines. This marked decondensation is not necessarily due to the lack of P2, but could also be produced by an increased proportion of intermediate proteins or of proteins migrating at the level of histones. The simple overall reduction in the levels of total protamine (due to the decrease in P2), which is not compensated by an increase of P1, could also contribute to the lack of condensation. Decondensed or unstable sperm nuclei in infertile patients have been reported (Ensou et al., 1989; Bustos-Obrégon and Leiva, 1983; Colleu et al., 1988; Dadoune et al., 1988; Blanchard et al., 1990; Jager, 1990; Auger, 1990; Schill, 1991; Pasteur et al., 1992). It will be interesting to determine whether there is a correlation or coincidence of this type of phenotype (selective absence of protamine P2 family) with previously described types of sperm based on morphological, dynamic, clinical, or cytogenetic considerations (such as globozoosperma, spermatid arrest, or chromosomal aberrations) (Navarro et al., 1990; De Braekeleer and Dao, 1991; Schill, 1991; Bibbins et al., 1992; Meyer et al., 1992). It will be equally interesting to determine if the physiological decondensation of the sperm nucleus occurring post-fertilization in the egg is altered in nuclei selectively lacking P2 by mimicking the decondensation process in vitro through incubation of the sperm cells with egg extracts or other agents (Huret, 1986; Brown et al., 1992; Lokha and Masui, 1983).

REFERENCES

Adroer, R., Queiralt, R., Ballabriga, J., and Oliva, R. (1992) Nucleic Acids Res. 20, 609.
Aiman, G., Griffin, J. E., Gazak, J. M., Wilson, J. D., and MacDonald, P. C. (1979) N. Engl. J. Med. 300, 223–227.
Arkhis, A., Martigné, A., Sautière, P., and Chevaillier, P. (1991) Eur. J. Biochem. 200, 387–392.
Auger, J., Mesbah, M., Huéber, C., and Dadoune, J. P. (1990) Int. J. Androl. 13, 462–462.
Bach, O., Glauden, H.-J., Scholz, G., and Schwarz, J. (1990) Andrologia 22, 217–224.
Balhorn, R. (1989) in Molecular Biology of Chromosome Function (Adolph, K. W., ed.) pp. 365–395, Springer-Verlag, New York.
Balhorn, R., Weston, S., Thomas, C., and Wyrobek, A. J. (1984) Exp. Cell Res. 159, 298–305.
Balhorn, R., Reed, S., and Tanphaichit, N. (1988) Experience 44, 52–55.
Belokopytova, I. A., Kostyleva, E. I., Tomilin, A. N., and Vorob’ev, V. I. (1993) Andrologia 25, 33–57.
Bianchi, F., Rousseaux-Prevost, R., Sautière, P., and Rousseaux, J. (1992) Biochim. Biophys. Acta 1120, 140–147.
Bibb, S. T., Hakanen, J. A., Ward, J. B., Legator, M. S., and Lipshultz, L. I. (1992) Fertil. Steril. 57, 402–408.
Boucher, G., Lurcat, J., and Leblond, D. (1989) Andrologia 22, 549–555.
Bower, P. A., Yeluc, P. C., and Hecht, N. B. (1987) Biol. Reprod. 37, 479–488.
Brown, D. B., and Nagamani, N. (1991) J. Biol. Chem. 265, 21059–21074.
Brown, D. B., and Nagamani, N. (1992) J. Biol. Chem. 265, 1826, 1404–1407.
Brown, D. B., and Nagamani, N. (1992) J. Biol. Chem. 265, 1826, 1404–1407.
Brown, D. B., and Nagamani, N. (1992) J. Biol. Chem. 265, 1826, 1404–1407.
Bustos-Obrégon, E., and Leiva, S. (1983) Andrologia 15, 488–478.
Cai, J. M., Rivas, F., Hernandez-Jaureguia, P. D., Diaz, M., Cortés-Gallegos, V., Vaca, G., Velazquez, A., and Ibarra, B. (1981) Hum. Genet. 59, 380–385.
Chapman, R. J., Jhabwar, S. C., Ehrenkranz, L. T., Couriades, I. A., and Williams, J. S. (1980) Am. J. Hum. Genet. 32, 833–848.

The intermediate proteins are a heterogeneous group of proteins that represent the protamine precursors at different stages of processing (Gusse et al., 1986; Sautière et al., 1988; Elsevier et al., 1991). Additional proteins could also be present at this level, such as transition proteins 1 and 2 (Luerssen et al., 1990; Baskaran and Rao, 1990; Schluter et al., 1992).
Protamine P2 Absence in Humans

Chauvière, M., Martinage, A., Debarle, M., Sautière, P., and Chevallier, P. (1992) *Eur. J. Biochem.* 204, 759-765
Chevailler, Ph., Mauro, N., Fenuex, J., Joannet, P., and David, G. (1987) *Lancer* II, 306-307
Collée, D., Lescat, D., Boujard, D., and Le Lannou, D. (1988) *Arch. Androl.* 21, 155-162
Dadonou, J. P., Minei, M. J., and Guhard-Moscato, M. L. (1988) *Andrologia* 19, 433-447
D de Brakteleer, M., and Dao, T. N. (1991) *Hum. Reprod.* 6, 245-250
Domenjoud, J., Frome, C., Uthe, F., and Engel, W. (1988) *Nucleic Acids Res.* 16, 1735
Domenjoud, L., Nussebaum, G., Adham, I. M., Greenske, G., and Engel, W. (1990) *Genomics* 5, 127-133
Domenjoud, L., Kremling, H., Burleind, P., Maier, W. M., and Engel, W. (1991) *Andrologia* 23, 333-337
Eisevies, S. M., Noirin, J., and Carre-Eusebe, D. (1991) *Eur. J. Biochem.* 196, 167-170
Evenson, D. P., Darzynakiewicz, Z., and Melamed, M. R. (1980) *Science* 210, 1131-1133
Flörke-Heidolf, S., Ting-Peter, E., Muler-Estel, W., Mansouri, A., Schatz, R., Schirren, C., Schill, W. B., and Engel, W. (1984) *Andrologia* 16, 187-202
Frage, C. C., Motchnak, P. A., Shigenaga, M. K., Helbock, H. J., Jacob, R. A, and Ams, B. N. (1991) *Proc. Natl. Acad. Sci. U.S.A.* 88, 11003-11006
Guise, M., Sautière, P., Bélache, D., Martinage, A., Roux, C., Dadonou, J. P., and Chevallier, P. (1990) *Bioclin. Biophis. Acta* 884, 124-134
Hecht, N. B. (1989) in *Molecular Biology of Chromosome Function* (Adolph, K. W., ed) pp. 396-420, Springer-Verlag, New York
Huret, J. L. (1986) *Arch. Androl.* 18, 97-129
Jager, S. (1990) *Arch. Androl.* 25, 233-259
Jegou, B., Veies de la Calle, J. F., and Bauché, F. (1991) *Proc. Natl. Acad. Sci. U.S.A.* 88, 8716-8717
Kasinsky, H. E. (1985) in *Histones and Other Basic Nuclear Proteins* (Hnilica, L., Stein, G., and Stein, J., eds) pp. 73-183, CRC Press, Boca Raton, FL
Kremling, H., Reinhardt, N., Schirren, M., and Engel, W. (1992) *Bioclin. Biophis. Acta* 1132, 133-139
Lee, C. H., Hoyer-Fendri, S., and Engel, W. (1987) *Nucleic Acids Res.* 15, 7639
Lee, C. H., and Balhorn, R. (1991) *Mol. Reprod. Devel.* 30, 154-158
Lescat, D., Collée, D., Boujard, D., and Le Lannou, D. (1988) *Arch. Androl.* 20, 35-40
Lobka, M. J., and Masul, Y. (1983) *Science* 220, 719-721
Luanstien, M., Mattéi, M.-G., Schröter, M., Grzeschik, K.-H., Adham, I. M., and Engel, W. (1990) *Genomics* 5, 302-306
Maier, W. M., Nussebaum, G., Dadonou, L., Klemm, U., and Engel, W. (1990) *Nucleic Acids Res.* 18, 1249-1254
McKay, D. J., Renaux, B. S., and Dixon, G. H. (1985) *Biosci. Rep.* 5, 383-391
McKay, D. J., Renaux, B. S., and Dixon, G. H. (1986) *Eur. J. Biochem.* 156, 5-9
Meyer, J. M., Maes, J. L., and Rumpler, Y. (1992) *Histopathology* 21, 25-33
Mizuta, C. (1985) in *Chromosomal Proteins and Gene Expression* (Keck, G. R., Goodwin, G. H., and Puigdomenech, P., eds) pp. 315-332, Plenum Press, New York
Navarro, J., Templado, C., Benet, J., Lange, R., Rajmil, O., and Egoscue, J. (1990) *Hum. Reprod.* 5, 227-229
Oliva, R., and Dixon, G. H. (1989) *J. Biol. Chem.* 264, 2472-2481
Oliva, R., and Dixon, G. H. (1991) *Prog. Nucleic Acid Res. Mol. Biol.* 40, 25-94
Oliva, R., Banett-Jones, D., Mezquita, C., and Dixon, G. H. (1987) *J. Biol. Chem.* 262, 17016-17025
Oliva, R., Goren, R., and Dixon, G. H. (1989) *J. Biol. Chem.* 264, 17672-17680
Oliva, R., Banett-Jones, D. P., Lockhart, L., and Dixon, G. H. (1990) *Nucleic Acids Res.* 18, 2739-2747
Plesch, X., Maggio, J., Schütte, G., and Laurent, J. L. (1992) *Anal. Quant. Cytol. Histol.* 14, 98-104
Pavlov, S. N., Brwer, K., Farley, M. G., Lindner, J., Bastias, M. C., Rogers, B. J., Swift, L. L., Niviet, J. E., Vale, W. W., Conn, P. M., and Herbert, C. M. (1991) *J. Clin. Endocrinol. Metab.* 73, 1360-1369
Pirzonen, A., Valtonen, P., Linnans-Kankkunen, A., Heikasen, M., and Haen-paa, P. (1990) *Bioclin. Biophis. Acta* 1039, 177-180
Poccin, D. (1986) *Int. Rev. Cytol.* 105, 1-65
Querelle, R., and Oliva, R. (1991) *Nucleic Acids Res.* 19, 5786
Retief, J. D., Winkfein, E. J., Dixon, G. H., Adroer, R., Querelle, R., Ballabriga, J., and Oliva, R. (1993) *J. Mol. Endocrinol.* 36, in press
Sautière, P., Bélache, D., Martinage, A., and Lorr, M. (1984) *Eur. J. Biochem.* 144, 121-125
Sautière, P., Martinage, A., Bélache, D., Arkhis, A., and Chevallier, P. (1988) *J. Biol. Chem.* 265, 11059-11062
Scaglia, H. E., Carrere, C. A., Mariani, V. A., Zolbergstein, C. C., Rey-Valschechi, G. J., Kelly, E. E., and Aquilano, D. R. (1991) *J. Androl.* 12, 273-280
Schill, W. H. (1991) *Hum. Reprod.* 6, 983-987
Schlegel, P. N., Chang, T. S. K., and Marshall, F. F. (1991) *Fertil. Steril.* 55, 235-242
Schletter, G., Kremling, H., and Engel, W. (1992) *Genomics* 14, 377-383
Snyder, P. J. (1990) *N. Engl. J. Med.* 323, 64-66
Staker, L. H., McKeeson, C., Balhorn, R., Lee, C., Mrazinas, J., Goralka, M., and Wryobek, A. (1992) *Mol. Reprod. Dev.* 33, 481-488
Tschussel, S. M., and Hecht, D. B. (1989) *Nucleic Acids Res.* 17, 4399
Tobita, T., Tsumori, H., Kato, A., Suzuki, H., Nomoto, M., Nakano, M., and Ando. T. (1983) *Bioclin. Biophis. Acta* 744, 141-146
Ueda, H., Yaya, M., Morit, N., Doi, Y., and Fujimoto, S. (1991) *Arch. Histol. Cytol.* 54, 401-410
van Beek and Meintrich, M. L. (1993) *J. Reprod. Fertil.* 94, 327-329
Winkfein, E. J., Nishikawa, S., Connor, W., Oliva, R., and Dixon, G. H. (1991) *J. Cell Biol.* 115, 89a
Yehre, L., de, and Oliva, R. (1993) *Anal. Biochem.* 209, 201-203