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NATHALIE JOSSO, MD, PHD

Interview conducted by
Michael Chappelle
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INTRODUCTION

Nathalie Josso, MD, PhD, is a member of the INSERM Unité de Recherches sur l'Endocrinologie et la Génétique de la Reproduction et du Développement at Paris-Sud University. A pediatrician, intrigued by the intersex conditions, Dr. Josso concentrated her research on fetal sexual differentiation, and particularly anti-Müllerian hormone (AMH). The existence of this fetal testicular factor had been postulated by Alfred Jost in the 1950s. However, the nature of the "Müllerian inhibitor" remained unknown. Dr. Josso's pioneering research led to a comprehensive understanding of the fetal testicular factor that she named AMH. Her work has contributed to worldwide research on AMH for almost forty years and has helped establish the basis for the relentless search of further knowledge on the subject.

BIOGRAPHICAL SKETCH

Dr. Nathalie Josso was born and raised in Paris, France, and received her MD degree from Paris University in 1960. After completing her internship and residency at the Enfants-Malades Hospital in the early 1960s, she went on to develop her professional career as an investigator at INSERM and a consultant in pediatric endocrinology in top-level Parisian institutions. In order to expand her background in basic science, she obtained a degree in endocrinology with Alfred Jost at the Paris Science Faculty. In 1971, using the bioassay of Régine Picon, she was able to demonstrate that AMH is a macromolecule incapable of crossing a semi-permeable membrane and, therefore, not a steroid. Next, by showing that any mammalian fetal testis could trigger the regression of rat fetal Müllerian ducts, she demonstrated interspecificity. This advance enabled her to use bovine fetal testes, from which she dissected the seminiferous tubules and showed that only Sertoli cells secreted AMH. These achievements were recognized internationally with invitations to deliver the Lawson Wilkins Memorial Lecture in Baltimore in 1975 and to the Laurentian Hormone Conference in 1976. For the next step towards purification of AMH, she recruited Jean-Yves Picard, and together they managed to obtain a preparation of bovine AMH, from which they concluded that AMH was a glycoprotein dimer, linked by disulfide bonds. Finally, Bernard Vigier was recruited to prepare monoclonal antibodies to AMH and later went on to show that AMH is secreted also by granulosa cells of the ovary. The group purified AMH to homogeneity in 1984. In 1986, Dr. Josso founded the Unité de Recherches sur l'Endocrinologie du Développement, which she directed for twelve years. The next year, her group mapped the human AMH gene to chromosome 19, using bovine AMH cDNA, isolated by Dr. Picard. Further achievements were the molecular characterization of the Persistent Müllerian Duct Syndrome (PMDS) and the demonstration by Dr. Rodolfo Rey, a post-doctoral fellow from Argentina, that Sertoli cell AMH production is regulated at puberty by an intricate interaction between testosterone, follicle-stimulating hormone (FSH), and meiotic germ cells. Dr. Rey now continues to study AMH regulation in his own research lab in Buenos-Aires. The use of AMH as a clinical diagnostic tool in pediatric endocrinology was facilitated by the development of a serum immunoassay, published in the 1990 January issue of the *Journal of Clinical Endocrinology and Metabolism* by three independent groups led respectively by Dr. Josso in Paris, Dr. Donahoe in Boston, and Dr. Hutson in Melbourne. Commercial AMH ELISA kits are now widely used in assisted reproduction. Finally, Dr. Josso's group, together with Dr. Richard Cate, the investigator who had isolated the human AMH gene in collaboration with Dr. Donahoe, cloned the human AMH receptor type II and demonstrated that mutations in its gene result in AMH insensitivity, characteristic of one sub-type of PMDS. Furthermore, Chrystèle Racine, a PhD student, showed that AMH receptors are present on Leydig cells, where AMH exerts a repressive effect on cell differentiation and steroidogenic capacity. In collaboration with Dr. Joan Massagué, Lucile Gouédard, another PhD student in Dr. Josso's group, identified a type I AMH receptor and was the first to show that AMH signals through Smad proteins. In recent years, Dr. Josso has gone back to clinical research, her latest publication, presented also in a poster at the Boston meeting of the Endocrine Society, is a survey of DSD management worldwide. Dr. Josso has received numerous awards including the Grand Prix Alexandre Joannidès, awarded by the French Academy of Science; the Andrea Prader Prize; Prix du Rayonnement Français; and the Märta Philipson Award.

Table of Contents—Nathalie Josso, MD, PhD

Introduction iii

Biographical Sketch iii

I. FAMILY BACKGROUND AND EARLY YEARS 1

[time code]
[0:00:00]
On the birth and childhood of her father, Anatole Muhlstein, in the Warsaw Ghetto—father's career in the Polish Diplomatic Service—parents meet and marry in Paris—early education in New York during World War II—marriage to Dr. François Josso, professor of hematology and a hemophilia specialist–birth of their three children—on the support and encouragement of François Josso and his death in 1981.

II. A CAREER IN MEDICINE 1

[0:03:55]
Choosing medicine: father's influence—anticipating an academic medical career—early interest in endocrinology—deciding to enter the French research institute, INSERM—choosing pediatric endocrinology—early interest in disorders of sex development—publishing a first paper in the *Journal of Clinical Endocrinology and Metabolism*.

[0:13:00] 3
In the laboratory of Alfred Jost
Deciding to study basic endocrinology at the Paris Science Faculty—on the scientific stature of Alfred Jost—Dr. Jost's work on anti-Müllerian hormone.

[0:24:20] 5
Enfants-Malades/INSERM
Working in the department of Professor Jean Frézal—early studies of the effects of testosterone on Wolffian duct in organ culture—learning to do organ culture with Ilse Lasnitzki.

III. CHASING THE ANTI-MÜLLERIAN HORMONE 6

[0:30:52]
On the early state of the art in anti-Müllerian hormone research—the importance of finding the right technology—the necessity of technically perfect work—Régine Picon's organ culture technique.

[0:34:00] 6

Finding a source
The target organ: a rat fetal Müllerian duct—demonstrating non-species specificity with Régine Picon's test and human fetal testes—on the advantages of using calf fetal testes.

[0:37:40] 7

Purifying AMH
Determining whether or not AMH was a steroid— discovering that Sertoli cells secreted AMH—on requesting the radiotherapy unit to irradiate culture dishes—placing irradiated fetal testes close to the rat Müllerian duct—problems with purifying AMH: toxicity of the homogenate—Roger Guillemin makes an important suggestion—working with Jean-Yves Picard--on getting fetal testes from the slaughterhouse in Rouen—finding that the incubation medium contains AMH—fractionation procedures—on assigning Bernard Vigier to learn monoclonal antibody technology at the Pasteur Institute—three monoclonal antibodies against bovine AMH-- Bernard Vigier finds that the ovary makes AMH—on ovarian reserve and the practical value of AMH—promoting the functional value of AMH as a marker in pediatric endocrinology—Alfred Jost's reaction to the purification of AMH—Jean-Yves Picard shows AMH is on chromosome 19.

IV. MENTORING 11

[1:09:15]

On the mentoring style of Alfred Jost—the importance of starting young people off with something relatively easy that will give results one way or the other—on encouraging Bernard Vigier to present his results at an international meeting.

V. THE ENDOCRINE SOCIETY 12

[1:14:25]

Publishing in Endocrine Society journals—presenting a poster at the Boston meeting of the Endocrine Society—surveying physicians around the world regarding management of intersex disorders.

VI. CURRENT VIEWS ON ENDOCRINOLOGY 12

[1:15:34]

Having a fascination with the molecular mechanisms and biochemistry of endocrinology—on the overwhelming aspect of the field.

Index 14

Interview History 15

I. FAMILY BACKGROUND AND EARLY YEARS

Chappelle: Dr. Josso, would you please tell me a little bit about your family background?

Josso: My father, Anatole Muhlstein, was born in the Warsaw Ghetto, where he spent his childhood--my grandfather was a rabbi there. During his childhood my father didn't even know Polish; he spoke only Yiddish. He spent all his time studying the Torah. He grew tired of that when he was about fourteen and he left the ghetto. He was more or less adopted by a Polish Catholic family, and then he followed normal studies. He went to university in Berlin and later was recruited into the Polish Diplomatic Service. He was sent first to Belgium and then to Paris, where he met my mother and they married. My father, after the war, was unable to go back to Poland. Since Poland was then Communist, he thought that he would not be welcomed there, and so he stayed in France.

Chappelle: What was it like for you and your family during World War II and its aftermath?

Josso: We left France because my father--being a diplomat--knew what was happening to Jews in Europe. He persuaded my mother's parents to leave, too, because they felt it was unpatriotic, and they should stay there-- my mother's brothers had fought in the war and had been taken prisoners, so my grandmother didn't want to leave. But my father was very persistent, and he took us all to the States. I spent all the war years in New York, and that's where I learned to speak English. And we went back to France in 1946.

Chappelle: What kind of education did you have?

Josso: When I was in New York, I went to an American public school for a year. I didn't know a word of English at that time--but okay, I learned. Then after that I went to the French lycée in New York. When we went back to Paris, I went to a lycée--a lycée is a public high school. From that I went to university, because in France we don't have college. After high school, you go straight to university--medical school in my case.

In 1959, I married Dr. François Josso, a professor of hematology and specialist in hemophilia, and together we had three children. François died of cancer in 1981, three years before the purification of AMH, a goal to which he had greatly contributed by his unflinching support and encouragement.

II. A CAREER IN MEDICINE

Chappelle: What drew you to medicine as a career?

Josso: I don't really know. As far as I remember, it was always understood that I

was going to study medicine. Retrospectively, I don't know whether it was my father's idea, or mine, too. But it was never questioned--even very early. I took up Greek in school; at that time future doctors were supposed to know Greek because so many words in medicine have a Greek etymology. I just entered medical school without thinking anymore about it and without considering any other options.

Chappelle: What kind of physician or physician-scientist did you think you would be?

Josso: I didn't at all think that I would be an investigator. I thought that I would care for patients in a hospital setting. I didn't feel that I wanted to open a private practice. I found a department where I felt comfortable and it was understood that I was going to be an assistant professor and, after that, a full professor with a normal academic career, caring for patients in hospital in the morning and teaching the rest of the time. But all this planning went wrong when I had finished my residency--I was more or less free in the afternoon--so I thought maybe I could go to the Science Faculty and learn some basic endocrinology. After a year as a student, I then took up research for a PhD and decided to forgo an academic career and enter INSERM (*Institut National de la Santé et de la Recherche Médicale)*, the medical research institute. Does that answer your question?

Chappelle: Yes. But I would like to know more about what got you interested in endocrinology?

Josso: I just happened to like it. In France, to be a resident with responsibility, one has to pass a rather difficult competitive examination, and I was lucky enough to get it the first year. I didn't expect that I would become a resident so early. And I hadn't much experience in the hospital. People, were telling me, "Oh, you're a woman, you're going to do pediatrics." And I thought "Why? I don't see why I should enter pediatrics because I'm a woman. I'm going to try different departments." One of the departments was endocrinology. I thought that very interesting. I'm not very good with my hands or mechanical things, and endocrinology is more intellectual in a way than some other specialties. So I liked endocrinology. But next I spent six months in a hospital for infectious diseases called Claude Bernard Hospital. I spent two months in what was considered the most interesting section, where you had adult patients with fevers, and you had to figure out what was the matter with them. The rest of the time you cared for measles, whooping cough--I had never done any pediatrics--I found myself with these children, and I decided, "that's what I want to do." Since I had enjoyed endocrinology--and now I liked pediatrics--I thought a good solution is to become a pediatric endocrinologist

Chappelle: What led to your interest in disorders of sex development?

Josso: Because of a patient. I was assigned an outpatient clinic in pediatric endocrinology. I had a patient who was a sixteen-year-old adolescent--raised as a boy--who suddenly developed breasts and bleeding from his urethra. He had been cared for by urologists because he had very severe hypospadias for which he had been operated many times. And when his breasts developed and he started to bleed, the urologist told him "You're a woman! We are going to make you into a woman!" Obviously, he was devastated, and his parents decided to send him to a medical department and find out what is the matter with him. It happened that I became responsible for this patient. I worked him up, and we discovered that he had an ovary on one side and a testis on the other. He had a bilateral mastectomy and corrective surgery. Our cytogeneticist, Jean de Grouchy found that he was a XX/XY mosaic due to double fertilization. I grew very interested in this case and published it in *JCEM (Journal of Clinical Endocrinology and Metabolism*).

[Interruption]

In the laboratory of Alfred Jost

Josso: It's difficult to specialize in a very rare disorder--activists would jump on me if they heard me say disorder because they pretend DSD (disorder of sex development) is just a third sex. Most of my patients were short or obese or had thyroid insufficiency, but I was most interested in those that had disorders of sex development. People knew it, and they sent these patients to me. And when I went to the Science Faculty to study basic endocrinology, the professor happened to be--I didn't choose him--it just happened to be Alfred Jost, who had made very important discoveries in the field of sex differentiation. So it all fitted in very well. I was interested in the clinical aspects of sex differentiation, and I was going to do research work with someone who was a very important person in that field.

Chappelle: Is the Science Faculty a separate institution or was that part of--it wasn't part of the hospital.

[Interruption]

Josso: No. There is a medical faculty linked to the hospital. The Science Faculty is something completely separate, like the law faculty for instance.

Chappelle: I see. Would you say a little bit about the scientific stature of Professor Jost?

Josso: Professor Jost was a giant. He was a physiologist. He had made extremely important discoveries on the dual nature of fetal testicular secretion. He had a big lab--he was interested in all aspects of fetal physiology--so some people were studying the fetal adrenals, others fetal thyroid or fetal liver. But paradoxically, he didn't have a section of his lab specialized in sex

differentiation because he wanted to keep it to himself. In his lab, of course, everyone knew about anti-Müllerian hormone--he didn't call it that; he called it *l'hormone inhibitrice,* the inhibitory hormone--he was, of course, best known for that. But no one was actually assigned to that research subject because it was *his* subject. He only had two junior people, PhD students, whom he employed because he was interested in freemartins. These young investigators went to the veterinary faculty and brought back freemartin gonads. But they were the only two, and they worked directly under Professor Jost. No one in the rest of the lab would dream of asking to work on anti-Müllerian hormone.

Chappelle: I'd like you to say a little bit more about how he found out about anti-Müllerian hormone and the other hormones that he worked with.

Josso: His main discoveries, those for which he is best known, he made while he was studying for his PhD thesis under Robert Courrier. At that time, no one knew which hormone was responsible for the regression of Müllerian ducts because none of the experiments that had consisted in removing fetal testes had resulted in the persistence of the Müllerian ducts. The old literature states that fetal testes have nothing to do with the regression of Müllerian ducts. Jost's idea was that probably the castration had not resulted in persistence of Müllerian duct because it had been performed too late. In fetal physiology there are windows of sensitivity: if you perform an experiment *before*--it will be ineffective; if you do it *after*--it will be ineffective, too. Jost developed a technique for castration of fetal rabbits, and initially he--like the others--got no results because he had castrated the fetal rabbits too late. Then he managed to do the experiment at an earlier time, and there he found that the rabbit fetuses from which he had removed the gonads developed as females; that meant that the Wolffian ducts regressed and the Müllerian ducts persisted. After that he went on to show that the fetal testis was making two hormones: one was the well-known androgen testosterone. However, when Jost implanted a testosterone crystal into a castrated rabbit, the Müllerian ducts did not regress, which meant that testosterone secreted by the fetal testes could not be responsible for the regression of the Müllerian duct. Therefore, he concluded in his thesis and in a later paper, which he presented at the Laurentian Hormone Conference, that there was a second hormone, which he called *l'hormone inhibitrice*. In the States, you often call it Müllerian inhibiting substance, that's what it's called if you look in the Endocrine Society program. I wanted to find posters or communications on AMH. I looked under anti-Müllerian hormone, and I couldn't find it. [laughs] So I looked under Müllerian inhibiting substance, and then I did. But there Jost stopped. He had demonstrated that the Müllerian inhibitor was *not* testosterone, but he was unable to find out what it was. And that was because the technique he had for demonstrating the existence of AMH was so, so difficult. When he castrated a fetus, nine times out of ten the fetus died before any conclusion could be reached. So, obviously, it was not a technique that could be used routinely to investigate the nature of AMH.

During the three years I spent in Jost's lab, everyone talked about *l'hormone inhibitrice,* "What is it? What can it be"? Professor Jost told us, "I'm working on it; it's none of your business." [laughs] And so we were all left with this tantalizing idea of this *mysterious* substance just *awaiting* discovery. But I knew that by staying in Jost's lab I would *never* find out because I would *never* be allowed to work on it. So I decided to leave and to go back to the *Enfants-Malades* (Hospital for Sick Children)."

Enfants-Malades/INSERM

At that time, Professor Jean Frézal, who was the second in command of the medical genetics department where I worked, had opened a lab, which was separate from the hospital. It was an INSERM lab. He had the lab, but he didn't have anybody to work in it. So if someone wanted to work there, he was only too happy to accept. In fact, he had anticipated that I would continue in the Clinical Department, So when I asked to join his lab instead, he thought I was crazy. I think it's still the same--being a clinician is much more respected, you earn more money, people call you professor. It's a much more prestigious position. But I said, "I'm interested in this AMH. I don't care if I earn less money. I'm married. As long as I earn enough to pay a nanny to take care of the children that's all I'm required to do." [laughs] So he agreed and that's how I found myself back in the *Enfants-Malades*, having learned in Jost's lab the elements of research and some very important principles, but completely free to do what I liked. Professor Frézal gave me a technician, which I would never have got in Jost's lab. And, progressively, I was able to assemble a small team of people working with me on AMH.

Chappelle: You spent some time with Ilse Lasnitzki when you were working with Jost.

Josso: I just spent a month there. In Jost's lab, the first task I was given was to study the effect of growth hormone on bone. That meant taking the pituitary out of rats and getting them to survive to see what happened to their cartilages. Only--as I mentioned before--I'm extremely clumsy. Either the rats would survive, but they'd still have their pituitary, or they died. It was awful. After a year of that torture, one day I triumphantly waved a reprint. "Look! Look! There is no point in my continuing. Someone else has found the answer; it's IGF (insulin-like growth factor)." [laughs] I wasn't allowed to say that I didn't want to go on doing that kind of work because it was too hard--that was an excuse that he did not accept. If I couldn't do something at first, I had to go on until I succeeded or else leave the lab. So it never entered my head to tell him, "Look I'd like to change because it's too difficult." However, saying that there wasn't any point in going on because someone else had found the answer, that sounded reasonable. So he said, "Okay, then what would you like to do?" At that time I was interested in sex differentiation, I knew Müllerian duct was out of bounds--but there was the Wolffian duct. I thought "Maybe I could study the

effect of testosterone on Wolffian ducts in organ culture. I'm definitely *not* castrating any fetal rabbits." I said, "Look, if you don't mind, I'd rather go and learn organ culture properly. There is a lady called Ilse Lasnitzki in Cambridge who does that very well." He accepted. But I just spent a month. I remember my daughter was just a baby. I was furious because when I left she was crawling, and when I came back she was walking. So I had missed her first steps while doing my first steps in organ culture. Well, I suppose you have to choose.

III. CHASING THE ANTI-MÜLLERIAN HORMONE

Chappelle: When you began your research on AMH, what was known about it?

Josso: Well, nothing except that it was different from testosterone and that it was made by the fetal testes. But I had a tool--which I did not develop myself. That was one very important thing you learned in Jost's lab. He used to say, "Everyone can have ideas, but not everyone can carry them out." Having ideas was fine, but if you did not have the technology your idea was worthless. Also, he had no patience for work that was not perfect. For instance, if you did histology, you couldn't just show him something that was torn and say, "Well, the section is torn, but nevertheless you can see what I mean." No, no, no. Everything had to be technically perfect. And that I think was very important for me because later on, when I had results, I looked at them and I thought, "Would I show them to Professor Jost? I mean, are they clear enough?" Because sometimes, when you are doing the work yourself, you may think, "Oh well, maybe it's not absolutely evident from my data, but I know that the conclusion is right." That kind of reasoning did not go down with Jost. In his lab, an assistant professor called Régine Picon had shown that if you culture a fourteen-day-old fetal rat reproductive tract--alone, without the gonads--the Müllerian duct will persist. And if you put fetal testes in--she was using a rat reproductive tract so she put in rat fetal testes--then the Müllerian duct will regress. So when I went back to the *Enfants-Malades*, I took Régine's technique back with me.

Finding a source

Chappelle: What were your initial steps?

Josso: I wanted to find out what AMH was and to purify it. I had no biochemical background, so I thought that it would be very easy and that in two-years time I would have my hormone in a test tube. The target organ--"Régine's" rat fetal Müllerian duct--I had already. So what was going to be the source of the hormone? I knew that I wasn't going to get anywhere using rat fetal testes because they were much too small. Since I was working in a hospital, it was rather easy for me to go to the OB/GYN department and ask--at that time, the rules weren't so strict--if there were aborted fetuses, Usually when the

fetuses were very small, they just gave them to me. So I cultured the rat fetal Müllerian duct, and put human fetal testes--well, pieces, of course--close to it and found that I got regression. Of course, I had controls showing that, next to the adrenal or the ovary, the Müllerian duct persisted. That was my first step trying to find a source. But, obviously, I was not going to use human fetal testes routinely because they were not easily available. So I decided to use calf fetal testes because they were very large and you could get them in slaughterhouses. I was rather surprised that--when cows became old--they slaughtered them without caring whether they were pregnant or not.

[Interruption]

Purifying AMH

Chappelle: You were saying that you had your target organ and you had your animal model and you had demonstrated interspecificity.

Josso: That's right. So then I could start in earnest. First I wanted to characterize AMH. At the beginning, the only thing one knew--because of Jost's experiments--was that it was different from testosterone. But that did not rule out another steroid. In fact, Jost rather thought it was another kind of androgen. So my second step was to try to find out the size of the molecule. I did something extremely simple; I just put vitelline--an egg vitelline membrane--between the rat Müllerian duct and the fetal testis, and I checked that direct contact between the two organs was not necessary. As long as the membrane between them was permeable, the hormone could go through and induce the regression of the Müllerian duct. After that, I put a dialysis membrane that was permeable only to molecules up to 15,000 MW--that meant that steroids could go through, but any molecules that were larger than 15,000 could not. When I put this dialysis membrane then the testis was not able to induce the regression. So the conclusion was that AMH was a macromolecule. I suspected that it was a protein. But I had no proof of that. It could have been a lipid or something else.

Chappelle: What led you to believe that Sertoli cells secreted AMH?

Josso: I was open-minded about it. In the fetal testes you have germ cells--but they are not supposed to have any endocrine activity--and Leydig cells which produce testosterone. But since I had shown that AMH was *not* a steroid, I thought that perhaps the other cell line in the testes, the Sertoli cell, is responsible.

Chappelle: I see.

Josso: By using these calf fetal testes that were extremely large, you could dissect out the seminiferous tubules--leaving out all the Leydig cells--and put the

seminiferous tubules next to the rat Müllerian duct. And I did get regression. In the seminiferous tubules you have both germ cells and Sertoli cells. I didn't think germ cells were making AMH, but I had to prove it. In the hospital, there was a radiotherapy unit. I went there with my culture dishes containing the fragments of fetal testes and asked them to irradiate my explants. Imagine, now, going into a radiotherapy facility with culture dishes and asking them to fit them in between patients! After irradiation, I just let the fetal testes sit for about two to three days--to let the germ cells die out--and then I put the irradiated fetal testes close to the rat fetal Müllerian duct. And I saw that the rat Müllerian duct regressed quite nicely whether or not the germ cells were there. So by elimination, I thought Sertoli cells were producing AMH. But we really proved it only much, much later, when we got an antibody against AMH and could show that it stained the fetal Sertoli cells.

Chappelle: After you had the Sertoli cells, did you have particular problems purifying AMH? What was your next step in purification?

Josso: Yes, we did have problems. For maybe a year or six months--I don't remember--I tried to homogenize the testicular tissue and put the homogenate in the culture medium in which I had the rat Müllerian duct. Normally you culture the rat Müllerian duct in the synthetic culture medium with fetal calf serum added. And, of course, in the absence of a fetal testis, the Müllerian duct will persist. So I reasoned that if I put homogenate containing AMH in the culture medium, then the Müllerian duct would regress. Only it didn't happen that way because the AMH is present in a very, very low concentration in testicular tissue, and you are limited in the amount of homogenate you can put in the culture media: if you put too much, it's toxic and three days after there is nothing left of the reproductive tract. And even putting the maximum amount of homogenate tolerated by the Müllerian duct--even then--the concentration of AMH in the culture medium was too low to have any effect. So we were stuck. I tried ultracentrifugation to try to keep only the cellular organelles that I thought would contain AMH--it was maybe a little better--I could put a little more homogenate, but still I couldn't put enough to get a result. And then I was invited to the Laurentian Hormone Conference--at that time it was in Canada, in a nice setting, and you could talk to other people. The star speaker that year was Roger Guillemin. Roger Guillemin, of course, has done all his career in the States, but he's French, so we talked. I was with Jean-Yves Picard, my co-worker, and we explained that we were having trouble because we couldn't show that homogenate contained our active substance. So he said, "I think you should try incubating your tissue and then test the incubation medium. Your hormone obviously is secreted in vivo; so in vitro, it will go into the medium, and the medium will not contain all the toxic structural proteins." So when Jean-Yves and I came back from the Laurentian conference, we decided to follow the Nobel laureate's advice. At that time we were getting our calf fetal testes from the slaughterhouse in Rouen--about 100 km from Paris, but there's a fast train connecting Rouen and Paris. We had arrangements with someone

working there that he would prepare a package containing the calf fetal testes at the end of the day--so we could have the day's collection--and then put it on the train. Then our technician would collect the package at the train station, would take the metro back to the *Enfants-Malades*, and there Jean-Yves and I would chop the testes into small pieces, put them to incubate under oxygen, and then three hours later--that was about 11 PM--we could stop the incubation, collect the incubation medium and freeze it. We did that twice a week; I saw a lot of movies instead of just waiting at home to go back to the lab at night. The medium did contain anti-Müllerian activity and could be used as starting material for different fractionation procedures,

Chappelle: Who is Bernard Vigier and what contribution did he make at this point?

Josso: Jean-Yves and I had been working very hard to fractionate the incubation medium. We had tried gel filtration, ultracentrifugation, ion exchange chromatography, and all the techniques we could possibly imagine. I had learnt histology in Jost's lab, and my job was to do the organ cultures with the different fractions Jean-Yves had prepared, look at the histology slide and say, "Yes, there's activity; no, there isn't." Just to make sure that I would not be influenced, the technician would code the slides, and I would say, "I'm finding activity in 'C' and there is no activity in 'B.'" After I had given my results in writing, I would be told what fraction they corresponded to. Jean-Yves was a geneticist; he had been mapping genes on chromosomes. Neither of us were biochemists. So we looked in books, we interviewed people, and we learned by trial and error but still--by gel chromatography--we could not identify a protein band directly connected with anti-Müllerian activity. We came to the conclusion that we needed a very, very, very specific technique--meaning, for instance, immunochromatography. If you have a specific antibody to the protein you want to purify, you stick that antibody on a column, you put your starting materials through the column, the protein of interest stays on the column because it is bound to the antibody, and all the rest flows through. Then when you wash the column, you put acid or anything you like to dissolve the bonds between the antibody and the protein, and your pure protein flows from the column to the test tube. The problem is that to be specific an antibody should be made against a purified protein, and that's just what we did not have. And then we learned about monoclonal antibody technology, which--given you have a specific test of interaction--will allow you to make a specific antibody against your protein of interest, even if you've immunized the mouse with a mixture. That is where Bernard Vigier comes in. You may remember that I mentioned that Jost had two young PhD students collecting freemartin fetuses for him: Bernard Vigier was one of them. He stayed in Jost's lab a long time, and he was interested in AMH. He thought that Jean-Yves and I were making progress, while Jost wasn't really working on the subject. So he decided to leave Jost's lab and to come to us. At the beginning I wasn't really very happy about that because I thought that Jost would be furious and would think that I had that I had--

Chappelle: Stolen?

Yes--that I had stolen Bernard from him. So first time around, I said to Bernard, "No. I think it would be very difficult. You can't leave Jost just like that. No." And then he came back the following year and I said, "Okay, but you have to make it clear to Jost that it wasn't my idea." But Jost was not furious at all. He understood that I had nothing to do with it, and I don't think that he held it against me. So when Bernard arrived, Jean-Yves and I told him, "You're now part of the group, but you must pay an entrance fee. Go to the Pasteur Institute and learn monoclonal antibody technology [laughs], and then come back and help us to purify AMH." And so poor Bernard, who had never, never done any immunology work in his whole life, went to Pasteur to learn the technique. We had a very, very insensitive technique to detect the wells that contained an antibody to AMH. But nevertheless, Bernard managed to create three monoclonal antibodies against bovine AMH, one of which was used to purify AMH to homogeneity in 1984. And then after that, Bernard made a very, very important discovery. He found that AMH is made by the ovary. Before that Jean-Yves and I had been working only on testicular AMH. But Bernard predicted that since Sertoli cells were making AMH; then probably granulosa cells were making it too, because there are many homologies between granulosa cells and Sertoli cells. So, he collected ovaries from cows and he equipped us all with syringes and needles. And he ordered, "You aspirate follicular fluid from these ovaries and make sure you separate the large follicles from the medium and the small ones." We protested he was crazy, but he insisted, "Just do as I tell you." So, Jean-Yves, myself, and Dien Tran, a Vietnamese electron microscopist--we did what he wanted. He had developed a radioimmunoassay against AMH, and he used it to show that the fluid from the small follicles contained a large amount of AMH, and the large follicles much less. Later, immunocytochemistry confirmed his findings. So in conclusion, Bernard was instrumental essentially in making the monoclonal antibody, which allowed us to purify AMH, and in showing that the ovary produces AMH.

Chappelle: Is that the ovarian reserve?

Josso: No. Bernard showed that granulosa cells produce AMH, but he didn't suggest any practical application. The concept that AMH is a marker of the number of follicles present in the ovary and all the work on the physiological role of AMH in the ovary--that is the work of a group in Rotterdam directed by Axel Themmen. Someone very active in that group is called Jenny Visser, and she was chairing a session this morning [at the Endocrine Society meeting in Boston] on follicular culture. Now, the practical value of AMH is recognized by gynecologists, and AMH is considered a very important tool in the assessment of patients undergoing in-vitro fertilization, but all the practical applications--they are the work of the Themmen's group. Rodolfo Rey and I

have tried to promote the use of AMH as a marker of the functional value of the infantile testes in pediatric endocrinology. Rodolfo has done a lot of work on that. But unfortunately that has not yet gained universal recognition.

Chappelle: When you had finally purified AMH, how was that work accepted? How did Professor Jost react?

Josso: Oh, he was overjoyed. But I think what really got him was when Jean-Yves mapped the gene for AMH on chromosome 19. The reason is that Jost's discovery of the existence of AMH was acclaimed by mostly doctors--pediatric endocrinologists in the States, for instance, Lawson Wilkins and Mel Grumbach--because his theories allowed them to explain a lot of the intersex disorders which they had seen. But in France, the recognized embryologist was Etienne Wolff, and he would not accept Jost's claim that there was a second hormone. For a long, long, long time, he maintained that the regression of Müllerian ducts induced by the fetal testes was due to testosterone. So Jost was contested by a whole school of fellow scientists. And the fact that he was considered a genius by pediatricians across the Atlantic didn't quite compensate for the lack of recognition by his peers. But when he was able to say, "AMH exists. Nathalie and Jean-Yves have got it in a tube, the gene is on chromosome 19," he was really extremely happy. You see Jost was an extremely intelligent person. He recognized that he had not taken the right steps to purify AMH. He could have got to it much earlier than we did. We had no biochemical expertise. Jost, in the Science Faculty, could have enlisted all the biochemists he wanted for the job. And he had the test also, since Régine Picon came from his lab. But he failed because he wanted no one else involved and he did not want to use a method developed by someone else. As I had trained in his lab he considered me his pupil. Is that what you say? In France we say, *son élève*.

Chappelle: Student?

Josso: Yes, his student. So if someone had to purify AMH--and it couldn't be him--might as well be me. [laughs] So we remained on excellent terms.

IV. MENTORING

Chappelle: Would you talk a little bit about your philosophy of mentoring, especially how you were mentored, and how you used that experience to help establish your own students?

Josso: Bernard and I used to remember our days in Jost's lab--we didn't have very good memories. Jost wasn't really helpful to young people. He considered that research was very difficult, that only the most intelligent, the most hardworking, the most persistent people would succeed. So he wasn't going to make it easy for you--as I explained with my pituitaries at the beginning. He

didn’t try to give young people something easy to begin with. He was also of the old school. When he went to meetings, he presented the findings of people in his group, but he presented them himself without mentioning his co-workers. Once someone from the lab was invited to a meeting and was given no help for his presentation. So, of course, it didn’t work out very well and he concluded, “When I don’t present the papers myself no one understands anything.” So when I had a lab of my own--of course I did not allow young people to do sloppy work--but I gave them, something relatively easy to start with, something that will give results one way or the other. Because some questions you ask are only informative--if you get one kind of result. If you get the other kind you cannot reach a conclusion. And also I was very, very careful not to imitate Jost and to always let my co-workers be senior authors and present their results themselves. I remember, once, Bernard and I went to a meeting in Sweden--and I said, “You’re going to present your results yourself." And he refused, "No, I won’t; I don’t speak English." Then when we were airborne, I told him, “Look, either this paper is not presented, or you do it yourself.” [laughs] He did quite well with a little help, and from then on he presented his own work. So, you see, I tried not to imitate Jost’s method of mentoring. But you have to place that in perspective because Jost was of the old school, and he wasn’t the only one to act like that. I don’t know if it’s the same in America, but in France and in Germany, the professor was all-powerful. He reigned over everything, and all the results that were obtained in his group were his, and the co-workers could only be grateful for being allowed to participate in the great work.

V. THE ENDOCRINE SOCIETY

Chappelle: What has been the nature of relationship with the Endocrine Society, and what have you looked forward to about this years meeting? I believe you are presenting a poster.

Josso: Much of my work has been published in the Endocrine Society’s journals, and this year I decided to present a poster on what I’ve been doing recently. I’ve been working on the management of intersex disorders by physicians in different parts of the world. So I and a Spanish colleague, Laura Audi, sent out a questionnaire to all the members of the European and Pediatric Endocrine Society. I’ve more or less written the paper, and I decided to present a poster. But of course if I hadn’t been coming for this interview, I would never have bothered to come to Boston just for that, it’s not that important.

VI. CURRENT VIEWS ON ENDOCRINOLOGY

Chappelle: What are your current views of the field?

Josso: Of what? Of DSD, or of the field of endocrinology in general?

Chappelle: Your choice.

Josso: I'm fascinated by the molecular and biochemical background underpinning modern endocrinology. It's fascinating, but in another way, it's rather overwhelming. It is very difficult to get a general view of all that's going on; it is difficult to keep up, but it's great fun.

Chappelle: Thank you.

[End of Interview]

Index—Nathalie Josso, MD, PhD

antibody, 8-10
anti-Müllerian hormone (AMH), 4-8, 10-11
Audi, Laura, 12
castration, 4, 6
chromatography, 9
chromosome, 11, 19
Claude Bernard Hospital, 2
Courrier, Robert, 4
de Grouchy, Jean, 3
dialysis membrane, 7
disorders of sex development (DSD), 2, 3, 11, 12
Endocrine Society, 4, 10, 12
endocrinology, 2-3, 12
Enfants-Malades (Hospital for Sick Children), 5-6, 9
fractionation procedures, 9
freemartin, 4, 9
Frézal, Jean, 5
gonads, 4, 6
granulosa cells, 10
Grumbach, Mel, 11
Guillemin, Roger, 8
hypospadias, 3
immunochromatography, 9
immunocytochemistry, 10
immunology, 10
infectious diseases, 2
INSERM (*Institut National de la Santé et de la Recherche Médicale*, "French Institute of Health and Medical Research"), 5
insulin-like growth factor (IGF), 5
interspecificity, 7
irradiation, 8
Jost, Alfred, 3-7, 9-10, 11-12
Journal of Clinical Endocrinology and Metabolism (JCEM), 3
Lasnitzki, Ilse, 5-6
Laurentian Hormone Conference, 4, 8
Leydig cells, 7
mentoring, 11-12
monoclonal antibody technology, 9, 10
Müllerian ducts, 4-8, 11
Müllerian inhibiting substance. *See* anti-Müllerian hormone (AMH)
Nobel Prize, 8
ovarian reserve, 10
ovary, 3, 7, 10
Paris Science Faculty, 2, 3, 11
Pasteur Institute, 10
pediatric endocrinology, 2, 11, 12
physiology, 3, 4, 10
Picard, Jean-Yves, 8-11
Picon, Régine, 6, 11
Polish diplomatic service, 1
radioimmunoassay, 10
residency, 2
Rey, Rodolfo, 10-11
seminiferous tubules, 7-8
Sertoli cells, 7-8, 10
sex differentiation, 3, 4, 5. *See also* disorders of sex development (DSD)
surgery, 3
testes, 3, 4, 6-9, 10, 11
testosterone, 4, 6, 7, 11
Themmen, Axel, 10
Tran, Dien, 10
urology, 3
Vigier, Bernard, 9-12
Visser, Jenny, 10
vitelline, 7
Warsaw Ghetto, 1
Wilkins, Lawson, 11
Wolff, Etienne, 11
Wolffian ducts, 4, 5, 6
World War II, 1

Interview History—Nathalie Josso, MD, PhD

Dr. Josso was interviewed by Michael Chappelle on June 4, 2011, during the Endocrine Society's Annual Meeting held at the Boston Convention and Exhibition Center in Boston, Massachusetts. The interview took place in a conference room at the Westin Hotel and lasted seventy-seven minutes. The transcript was audit-edited by Mr. Chappelle and reviewed by Dr. Josso prior to its accession by the Oral History of Endocrinology Collection. The videotape and transcript are in the public domain, by agreement with the oral author. *The original recording, consisting of two (2) 45-minute mini DV cam tapes, is in the Library holdings and is available under the regulations governing the use of permanent noncurrent records*. Records relating to the interview are located in the offices of The Clark Sawin Library's Oral History of Endocrinology Project.