#211: Sexing the wallaby: Marsupial reproduction and what it says about us

VOICEOVER
Welcome to Up Close, the research talk show from the University of Melbourne, Australia.

DYANI LEWIS
I'm Dyani Lewis. Thanks for joining us. Each and every one of us started out life as a simple fertilised egg. A cascade of precisely coordinated genetic programs propels the developing embryo through growth and differentiation in preparation for birth. Getting to grips with these complex processes has been the preserve of developmental biologists and has resulted in a clutch of Nobel Prizes for the field. Some of the leaps and bounds that developmental biology has taken over the last century are owed to the concerted studies of a few key species or model organisms as they are known. Fruitflies, Zebrafish, frogs and mice have all taught us something about how complex bodies develop from fertilised eggs. Our guest on Up Close today has taken a somewhat different path. Instead of focusing his efforts on these traditional model organisms, Developmental Biologist Geoff Shaw, has been working on the Tammar Wallaby, a small member of the kangaroo family of marsupials. In doing so, he has learned not only about the fascinating reproductive lives of wallabies, but also about sex determination in humans. Geoff Shaw is Professor Developmental Biology in the School of Zoology at the University of Melbourne. Welcome to Up Close Geoff.

GEOFF SHAW
Thank you.

DYANI LEWIS
No for listeners who may not have heard of a wallaby before, could you describe for us what a wallaby is?

GEOFF SHAW
Small marsupials. In fact members of the kangaroo family which range up to the Red Kangaroo, which I'm sure everyone's familiar with. Hop along on the back legs, have a pouch with a baby. Think a small kangaroo.
And the wallaby, as with other kangaroos and also quite a few other Australian mammals, are what's known as marsupials. So what does this mean and how closely are marsupials related to other mammals, the placental mammals?

GEOFF SHAW
So the early mammals arose from a reptilian ancestor and took three branches. One that led to the monotremes which are remarkable egg laying mammals that we have in Australia and another branch that went to the marsupials and the third branch went to eutherian or placental mammals. The marsupials are fascinating because of their embryonic development. So they give birth to babies that are - in eutherian mammal terms - are foetal. So a lot of the development that happens after they are born mirrors changes happening in foetuses in placental mammals or eutherian mammals.

DYANI LEWIS
What are some of the problems that this really early birth presents for marsupials?

GEOFF SHAW
I don't know that it presents necessarily any great problems. It means that the delivery of nutrition to the babies is a little bit slower than you can achieve through a placenta. They're drinking milk. But again they've adapted their lactation, a fascinating story all by itself. So they produce milk of different concentrations to suit the different needs of young at different ages, to suit their embryonic development stages.

DYANI LEWIS
Okay so how does it change over that developmental time?

GEOFF SHAW
So the early milk is very dilute, low in fat and contains the nutrients needed for the developmental stage that they're at, at that stage. Very poorly developed lungs, very poorly developed kidneys, the gut's hardly formed and so everything needs to be easy to digest and processed by the body. By the time they're close to leaving the pouch the milk is extraordinarily rich in fats, very concentrated, high energy and contains specific fats that are useful for example for building brains, which is happening very much at the later stages of lactation.

DYANI LEWIS
What sizes are we talking about here when the wallaby is born and crawls into the pouch? How big is it and the how big is it when it leaves the pouch?

GEOFF SHAW
Okay I'll give you an example. Let's say a 50 kilogram woman gives birth to a three kilogram baby. So that's about six per cent of her body mass. The wallaby weighs five kilograms, that's 5000 grams and gives birth to a baby weighing half a gram.
Wow.

GEOFF SHAW
That's one ten thousandth. Then it grows. By the time it weans it's up around one and a half to two kilos, probably 2.2 kilos before they're fully weaned.

DYANI LEWIS
Could you describe for us the typical reproductive cycle of the wallaby?

GEOFF SHAW
Okay so they've got some special adaptations. Unlike most marsupials they're very strictly seasonal breeders, regulated by photoperiod. As day length starts to decrease after Christmas, it activates a reproductive cycle. They give birth about a month later in mid to late January. The females mate within a few hours of giving birth. So the baby climbs in the pouch, starts sucking on the teat, mum's mated, gets a fertilised egg which will develop to a 100 cell embryo, just a hollow ball of cells and it will stop developing. While the baby's sucking, during the first half of the year, that embryo will remain dormant. In the second half of the year, when the young might wean in September, October, November, but by that stage there's a photoperiodic, a day length mediated inhibition. So that embryo stays dormant until just after Christmas, decreasing day length in the Southern Hemisphere after Christmas, then gives rise to the reactivation. Birth in late January, mating again and a nice annual cycle.

DYANI LEWIS
This embryonic dormancy, does it happen in any other animals?

GEOFF SHAW
There's over 100 species of mammal, marsupial and placental eutherian mammals which are able to have that sort of thing, including mice, deer, bears and so on. Species from just about every major group of mammal have it. We think that this embryonic dormancy or diapause as it's called is probably something which is intrinsic in the biology and different species will activate it or inactivate it, depending on whether it makes sense in their life histories.

DYANI LEWIS
So there can be other triggers other than lactation and day length in wallabies for example that cause the dormancy in other animals, is that right?

GEOFF SHAW
In other animals it varies. Lactation is commonly a trigger. So in mice for example lactation is the trigger. In some species it's photoperiod. We haven't studied a lot of other species in great detail. So we don't actually know the regulatory causes. But I think probably photoperiod and lactation are the main ones.

DYANI LEWIS
Now we often contrast marsupials with placental mammals and I guess the more
correct term is eutherians. But does that imply that marsupials do not have a placenta?

GEOFF SHAW
Marsupials certainly have a placenta. It functions in much the same way as the placenta does in eutherian mammals as the early stages of gestation. So the eutherian mammals start off with a membrane called the yolk sac surrounding it which interacts with the lining of the uterus and provides nutrition to the early growing embryo. It's only later as the foetus gets bigger that the placenta that we are familiar with, the chorioallantoic placenta becomes important. So marsupials have a fully functioning placenta. It transfers nutrients and waste between the mother and the foetus and does everything that the placenta of the so called placental mammals does.

DYANI LEWIS
It just doesn't go on to form that more complex chorioallantoic placenta?

GEOFF SHAW
In one group of marsupials, the bandicoots, they in fact form a chorioallantoic placenta. All the more remarkable because they have among the shortest gestations of any mammal, 12 days or so, which is remarkably short.

DYANI LEWIS
That is very short. I'm Dyani Lewis. My guest today is Developmental Biologist, Professor Geoff Shaw and we're talking wallabies here on Up Close. Geoff you've been working on sex determination in wallabies. Presumably at the embryo stage of development, their 100 cell stage that you were talking about before, there's no way of telling a male embryo from a female embryo. I was just wondering when are we able to differentiate the two sexes in wallabies?

GEOFF SHAW
That's a fascinating story. The determination of sex in most mammals is a result of whether or not you've got one X chromosome and one Y chromosome or two X chromosomes. If you have two X chromosomes, you're genetically female and will develop a female body. If you're carrying an X from the egg and a Y chromosome from the sperm, then you're genetically male. But initially there's no difference between male and female embryos. The differences happen quite late in development with differentiation of the testes, or at least so we thought until we looked closely with our wallabies. The differentiation in humans up to about week eight is the same in males and females. In our wallabies it's about birth.

DYANI LEWIS
And so at birth, the young wallabies, you can't tell whether the gonads are going to be testes versus ovaries, is that correct?

GEOFF SHAW
Yes. So in mice and humans it's in the foetus that those indifferent stages transition
into male or female, in early pregnancy. In our wallabies, because gestation is so short, the testes or ovaries don’t differentiate until after birth.

DYANI LEWIS
And I guess this is one of the reasons that a wallaby is quite good to work on for this kind of problem.

GEOFF SHAW
Yes it allows us to look at the hormones made by the testes and see how they interact to drive male development and indeed what’s happening in the female in terms of development. But there’s one little twist. In the conventional model of sexual differentiation, it’s the hormones from the testes which drive all of the differentiation between males and females. So male development depends on the formation of the testes and the testes making hormones. In our wallabies, we noticed, Marilyn Renfree and Roger Short had an argument about this. Roger said there can’t be differences when they are born because the testes haven’t formed. Marilyn says, but I can see a difference.

DYANI LEWIS
Your biologist colleagues, Marilyn Renfree and Roger Short were discussing this because it was such an unusual observation wasn’t it?

GEOFF SHAW
Yes. And she could see that the male foetuses had little bulges that were forming later into the scrotum and the female foetuses had little skin patches that were developing into mammary glands and only in the genetic females could we see the mammary patches, only in the genetic males could we see the scrotal bulges. If we look back in embryonic development, those differences between male and female embryos were happening four days before birth. At that stage, the gonad is hardly formed; there is no testes, there was no ovary, there’s no hormones to drive that.

DYANI LEWIS
What’s creating and what’s generating that early development if not the male hormones?

GEOFF SHAW
It looks like it’s directly driven by the genetics and it seems that if you have two X chromosomes you’ll form the mammary buds. If you have an X chromosome, regards of whether you’ve got a Y chromosome, just one X chromosome, then you’ll form scrotal bulges. So two X’s is female and one X is male as far as the scrotum and mammary glands are concerned.

DYANI LEWIS
What are the implications of this observation then?

GEOFF SHAW
It means that not all sex differences are driven by the testes. And once we’d
observed this in our wallabies, we looked back through some old publications and even back in the 80s before we'd done our studies and there were studies showing that male foetuses, back at the very earlier stages, the blastocyst stage, before a foetus forms, could be discriminated roughly on the basis of size. So the embryos that grew fastest, tended to be male. Embryos that grew slowest tended to be female. There has been a number of studies since which have shown that there are other genetic influences besides those that make a testes which affect development. And it's interesting to speculate how many differences we see between males and females, many, many differences, how many of those are actually driven by the sex chromosomes irrespective of hormones.

DYANI LEWIS
Presumably the hormones are still very important in development though. So could you describe some of the work you've done on measuring those hormones in the young and what they are doing in the developing wallabies?

GEOFF SHAW
Certainly. The differences that are directly genetic are probably quite subtle. The major, the dominant changes in the body form are definitely driven by the hormones from the testes and later when the ovaries start to function by the ovarian hormones. It's very hard to actually study that in the eutherian mammals where those sex differences happen in early embryo development. By the time they're born, everything's pretty well finished. So we've been looking at the hormones in our wallabies and found that the logical hormone that you'd expect to be driving it, testosterone, isn't.

DYANI LEWIS
So that was quite a unique finding then?

GEOFF SHAW
Yeah, everyone assumed that testosterone would be the key hormone because that's the hormone that is made by the testes in the adult and maintains male development features. But in fact, in our wallabies, it's not testosterone. It's not the more active metabolite of testosterone called DHT. In fact there's a hormone called androstanediol. And androstanediol is inactive. But it's the hormone which circulates - it's made by the testes, circulates in the blood and when it gets to the target organs, the organs in the body which will be differentiated by testosterone, it's then converted into the active hormone DHT.

DYANI LEWIS
Now androstanediol had not been previously recognised as being so important. Why do you think that you stumbled upon it in the wallaby and no one else had recognised it?

GEOFF SHAW
Other people haven't had the advantage of working with an animal where they can look at the hormones after birth. It's very hard to study these things in a foetus.
DYANI LEWIS
The importance of male sex hormones in sex differentiation has led to many people describing females as the default sex. So if anything goes wrong, then a female results.

GEOFF SHAW
And that's a reasonable assumption. If the formation of testicular hormones or the action of testicular hormones is blocked or defective, then a female phenotype will result. It's not as simple as that because there are active pathways going on in female differentiation too. So someone who has insensitivity to male hormones or a total lack of male hormones is not exactly the same as a normal development.

DYANI LEWIS
So there's been quite a lot of attention in the media on sexual differentiation and the 2012 Games in London have been the first to use testosterone in gender testing of athletes. Do you think testosterone is a good way of, I guess, determining whether someone should be classified as a female for athletic purposes?

GEOFF SHAW
I think the picture is far more complicated than the Olympic Committee can allow. So I mentioned earlier that there can be differences which are not driven by the hormones at all, but are purely genetic. Those differences may or not be important for athletic performance, or at least some of those. It's very hard to say. There are athletes competing at the moment who have got a defective receptor for the male hormones. And so they're unable to respond to the hormones. They develop as females in body form, even though they've got a Y chromosome. But are they truly female in the same way that an XX female is? It's hard to say.

DYANI LEWIS
I'm Dyani Lewis and my guest today is Developmental Biologist, Professor Geoff Shaw. We're talking about sex determination in wallabies and what this means for human development, here on Up Close. Geoff, you were involved in the sequencing of the genome of the Tammar Wallaby. What has this told us about the reproductive biology of the animal?

GEOFF SHAW
Okay, the sequencing of the genome has given us a huge amount of resources which are allowing us now to much better understand the processes of developmental biology. Remarkably there's enormous conservation of the genes, despite the differences in developmental profile, between marsupials and eutherian mammals. They're very, very close on a genetic basis. And many of the processes are very conserved. What's important are both the similarities and the differences. Because we can match up the differences with differences in how the animals develop and how they live in a way that gives us a lot of power for understanding. Many people have assumed a function for certain genes because that gene turns on at that particular time in development. When we've looked in our marsupials, to match up the pattern of gene expression, sometimes you find a different pattern and
then we can say well this is happening in our wallabies, before the gene turns on, the gene isn't important.

DYANI LEWIS
Okay so separating those processes.

GEOFF SHAW
Yes, so we've got a very different time profile of development because our marsupials are born so early in development and it changes a lot of the gene expression patterns.

DYANI LEWIS
I wanted to come back to the Y chromosome in male development. The Y chromosome is very much smaller than the X chromosome. How does it create such a huge influence on development?

GEOFF SHAW
The Y chromosome is an interesting chromosome. It's developed its function as a sex determining switch. There's a process which has meant that it's lost most of the other genes that were on the original chromosome from which it formed. My colleague Jenny Graves has done a lot of work on understanding how those Y chromosomes come to be. She sometimes says that if you leave it long enough now the Y chromosome will totally disappear.

DYANI LEWIS
But surely you will still need that master switch to be there.

GEOFF SHAW
Yes and all you need is a switch, because what the gene on the Y chromosome called SRY for sex determining region of the Y chromosome, so SRY is a switch gene. It turns on a whole lot of genes on other chromosomes and those other genes turn on other genes and you get a whole cascade of developmental changes as a result. It's a bit like flicking a switch on a wall. It might just turn on a single light. It might turn on a big flashy neon display. It might turn on 10 bits of equipment. All you need is one little switch to supply the power. In the case of the Y chromosome, one little switch to turn on those other genes, that turn on other genes that lead to formation of a testes, making of hormones, or hormones do other things.

DYANI LEWIS
So if you have a faulty switch?

GEOFF SHAW
If you have a faulty switch, you don't get male development.

DYANI LEWIS
So you have an XY female, is that right?
GEOFF SHAW
So you're going to have XY females yes.

DYANI LEWIS
How well have we been able to apply what you have found in wallaby sex development to better understand our own development?

GEOFF SHAW
Well one example is our understanding of this hormone pathway involving androstanediol, which as I mentioned before has been previously thought to be an inactive hormone. When in fact now we know that this pathway to make androgens, via androstanediol is present. People have looked in mice and gosh, it's there too. We just hadn't thought about it before. We look in humans, it's there too. In fact now it's been recognised that that pathway is a pathway which operates in humans and variations in how it happens will lead to differences in male development. So a number of cases of improper male development in humans have been now explained by this alternate pathway for hormone formation.

DYANI LEWIS
Do marsupials then make quite good model organisms for medical research?

GEOFF SHAW
Yes they do. It's a question we are often asked in grant interviews or grant responses for the National Health and Medical Research Council, which funds our research quite handsomely. Yes they are. In fact the androstanediol story has now made it into the clinical guidelines for treatment of male sex differentiation anomalies.

DYANI LEWIS
Well thank you for being our guest on Up Close today, Geoff.

GEOFF SHAW
Thank you.

DYANI LEWIS
That was Geoff Shaw, Professor of Developmental Biology in the School of Zoology at the University of Melbourne, Australia. Relevant links, a full transcript and more info on this episode can be found at our website at upclose.unimelb.edu.au. Up Close is a production of the University of Melbourne, Australia. This episode was recorded on 22 August 2012. Our producers for this episode were Kelvin Param and Eric van Bemmel. The associate producer was myself, Dyani Lewis and audio engineering was by Gavin Nebauer. Up Close is created by Eric van Bemmel and Kelvin Param. Until next time, goodbye.

VOICEOVER
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