INTRODUCTION

This activity is based on the HHMI BioInteractive resource "Sex Verification in Athletes". The debate of how to categorize individuals as female versus male for the purpose of sporting events has long existed and was recently brought to light with the case of the elite female athlete, Dutee Chand, who has naturally occurring high testosterone levels. The question is: should females with high testosterone be allowed to compete as a female? The case of Dutee Chand is used as an engagement piece and the associated Click and Learn in the HHMI resource that accompanies this activity helps students discover how genes and testosterone regulate sex determination in humans.

In the activity, you will explore data in the form of a bar graph. Serum testosterone levels in female versus male elite athletes has been previously characterized (Healy, 2014). The data reveals a wide range of testosterone levels in both males and females and a great deal of overlap between the two biological sexes. After analyzing data representing typical serum testosterone levels in athletes, you will be asked to predict testosterone levels in individuals with differences in sexual development (DSDs) and compare your predictions to actual data. As such, you will build an understanding of biological sex determination, including how hormones regulate the development of sex characteristics, and thus phenotype. In so doing, you will also make the link between genetics and phenotype.

PROCEDURE/BACKGROUND INFORMATION

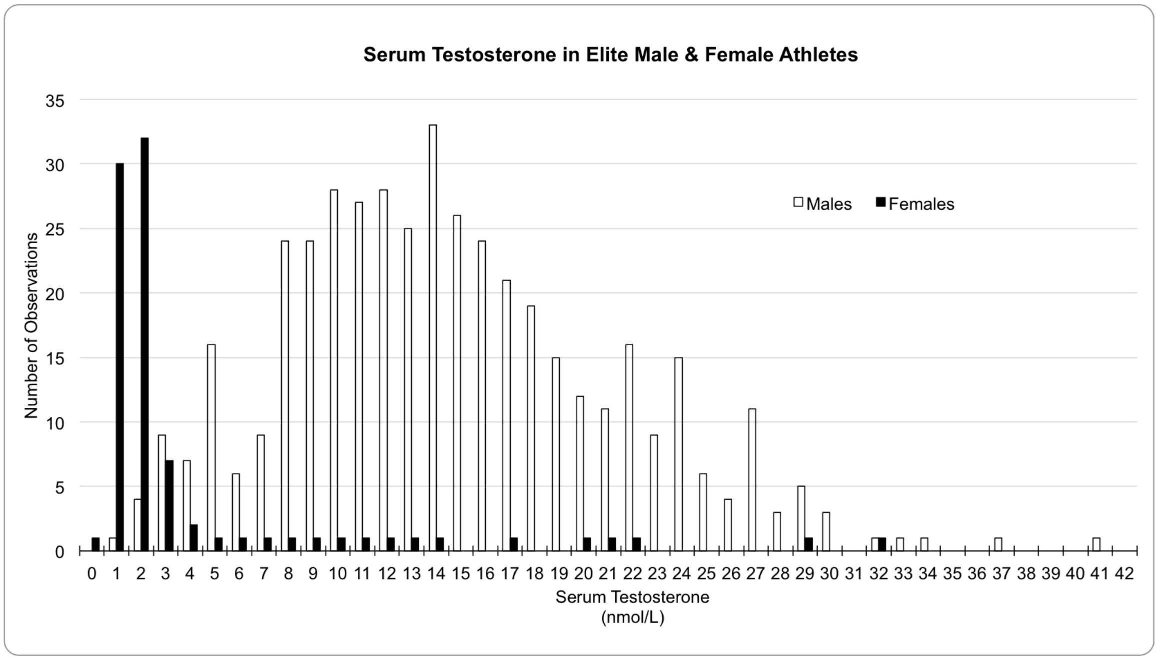
Watch the Introduction video from HHMI BioInteractive's "Sex Verification in Athletes". Your instructor may ask you to discuss the questions at the end of the video as a class or in a think-pair-share. You will then work in groups of 2-4 on one of four versions of the activity. The activity has been divided into four separate groups, each with a different DSD to explore. In your group, work together to analyze the graph and answer the questions in your Part 1. This part of the activity includes a prediction question. Once your group has made a prediction of the testosterone levels you expect in individuals with their DSD, your instructor will provide your group with Part 2 of the activity, including the actual data, to compare your prediction from part 1 and complete the questions. You may be asked to present your DSD to the class for discussion.

MATERIALS

- HHMI BioInteractive Click and Learn "Sex Verification in Athletes": Introduction video. https://www.hhmi.org/biointeractive/testing-athletes
- Platform on which to present video: computer, internet, projector.
- Print-outs of the group activities to hand-out to each student.

QUESTIONS

Group 1: Part 1



Graph adapted from Healy *et al*, 2014

1. Based on the graph above, describe the trends you see in the testosterone levels of elite males and female athletes. (Who has more? What is the distribution?)

2. What is the range of testosterone levels measured in the male athletes displayed in the graph? What is the range of testosterone levels measured for the female athletes? Do the ranges overlap? (Remember to use labels.)

3. a. If an individual had a serum testosterone level of 9 nmol/L, would you be able to <u>definitively</u> determine the individual's biological sex? Why or why not?

b. What would your prediction of the individual's biological sex be? Provide a scientific justification for your answer.

4. A number of different genetic variations can result in differences in sex differentiation. One example of this is a 46, XY female who is androgen insensitive. This means that the individual has a typical Y chromosome but has a mutation in their androgen (testosterone) receptors such that cells are unable to properly respond to testosterone.

a. Would these individuals have the SRY gene? Why or why not?

b. Would you predict that these individuals be able to produce levels of testosterone typical for biological males? Why or why not?

c. Looking at the range of testosterone levels from the graph above, predict an approximate serum testosterone level for a 46, XY androgen insensitive female elite athlete. (This estimate need not be exact.) Explain your reasoning.

Once you have completed the questions, obtain the Group 1: Part 2 sheet from your instructor.

Group 1: Part 2

Below are the actual serum testosterone levels were measured in 12 different 46, XY androgen insensitive females.

2.0 nmol/L	9.7 nmol/L	27.1 nmol/L
4.3nmol/L	10.8 nmol/L	35.1 nmol/L
8.3 nmol/L	12.4 nmol/L	35.4 nmol/L
9.4 nmol/L	20.1 nmol/L	54.1 nmol/L

Use the data above to answer the following questions.

d. What is the range of serum testosterone levels in the individuals above?

e. What is the average of the serum testosterone levels measured for these individuals?

f. How far off was your prediction from the average? Was it within the range?

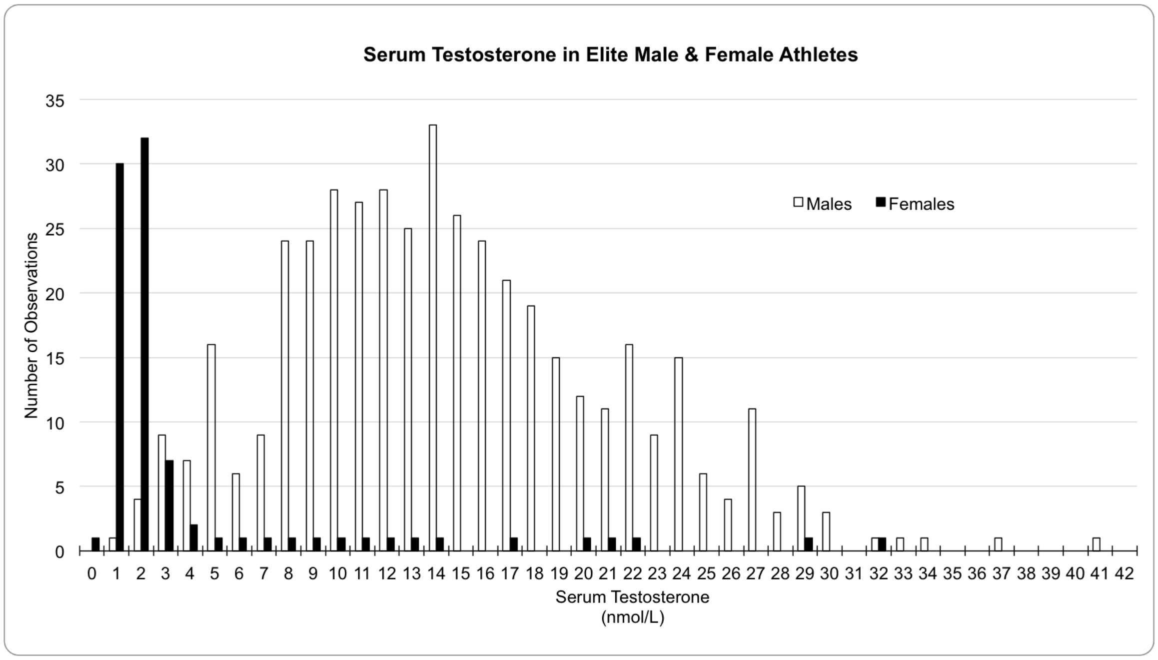
g. Given what you have learned, explain why 46, XY androgen insensitive females have this level of testosterone.

<u>References</u>

Healy ML, *et al.* (2014) Endocrine profiles in 693 athletes in the postcompetition setting. *Clinical Endocrinology.* **81**, 294-305.

46, XY androgen insensitive female testosterone levels from Chan et al (2015) Hong Kong Med J 12(6):499-510, Isurgugi et al (1996) Endocr J 43(5):557-564 and Melo et al (2003) J Clin Endocrinol Metab 88(7):3241-3250

Group 2: Part 1



Graph adapted from Healy *et al*, 2014

1. Based on the graph above, describe the trends you see in the testosterone levels of elite males and female athletes. (Who has more? What is the distribution?)

2. What is the range of testosterone levels measured in the male athletes displayed in the graph? What is the range of testosterone levels measured for the female athletes? Do the ranges overlap? (Remember to use labels.)

3. a. If an individual had a serum testosterone level of 9 nmol/L, would you be able to definitively determine the individual's biological sex? Why or why not?

b. What would your prediction of the individual's biological sex be? Explain your reasoning.

4. A number of different genetic variations can result in differences in sex differentiation. One example of this is a 46, XY female who has a mutation such that testosterone is not produced at levels typical of individuals with an SRY gene. This can be caused by mutations in the SRY gene itself or one of the other proteins that are induced by SRY that lead to testosterone production.

a. Given what you have learned, would you expect these individuals to have a Barr body in their cells? Why or why not?

b. Would you predict that these individuals be able to respond to the testosterone that is produced? Why or why not?

c. Looking at the range of testosterone levels from the graph above, predict an approximate serum testosterone level for a 46, XY female elite athlete with one of these mutations in SRY or other testosterone production proteins. Explain your reasoning.

Once you have completed the questions, get the Group 2: Part 2 sheet from your instructor.

Group 2: Part 2

Actual serum testosterone levels from 4 different 46, XY females with mutations in SRY or another gene involved in testosterone production are below.

3.9 nmol/L	10.3 nmol/L
9.6 nmol/L	13.6 nmol/L

Use the data above to answer the following questions.

d. What is the range of serum testosterone levels in the individuals above?

e. What is the average of the serum testosterone levels measured for these individuals?

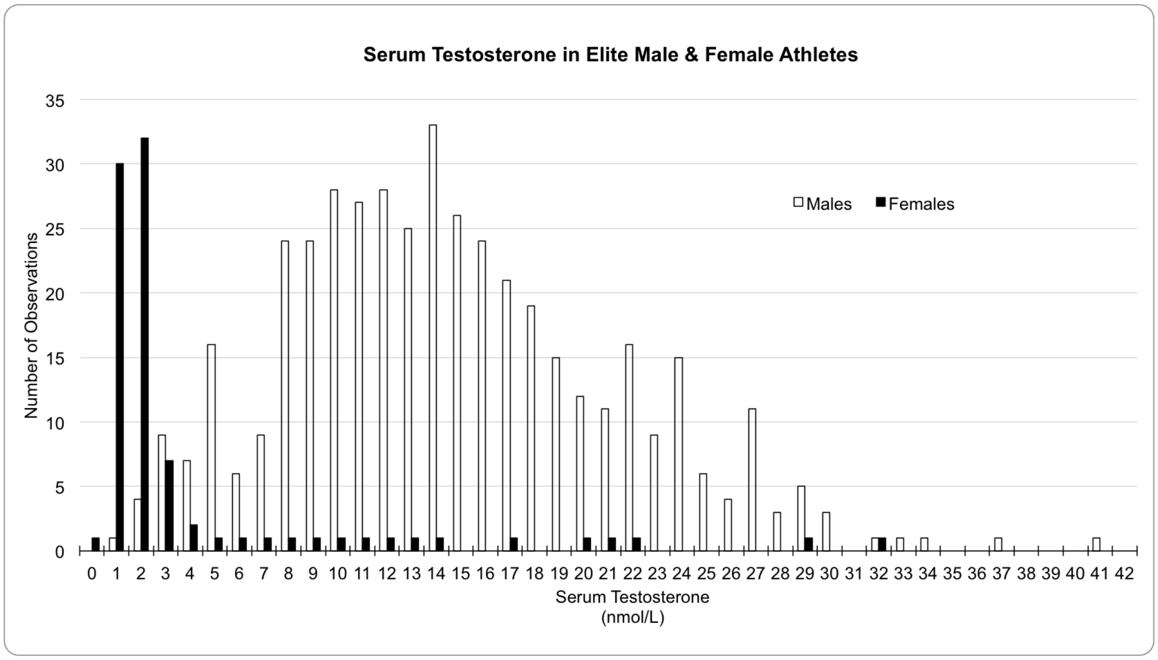
f. How far off was your prediction from the average? Was it within the range?

g. Given what you have learned, explain why 46, XY females with mutations in SRY or another gene involved in testosterone production have this level of testosterone.

References
Healy ML, et al. (2014) Endocrine profiles in 693 athletes in the postcompetition setting. Clinical Endocrinology. 81, 294-305.

46, XY female testosterone levels from Kulle et al (2012) Sex Dev 6(4) 161-168; Leme de Calais et al (2011) Int J Mol Sci 12(12): 9471-9480; and Sullivan et al (2018) J Clin Transl Endocrinol Case Report 7: 507.

Group 3: Part 1



Graph adapted from Healy *et al*, 2014

1. Based on the graph above, describe the trends you see in the testosterone levels of elite males and female athletes. (Who has more? What is the distribution?)

2. What is the range of testosterone levels measured in the male athletes displayed in the graph? What is the range of testosterone levels measured for the female athletes? Do the ranges overlap? (Remember to use labels.)

3. a. If an individual had a serum testosterone level of 9 nmol/L, would you be able to <u>definitively</u> determine the individual's biological sex? Why or why not?

b. What would your prediction of the individual's biological sex be? Explain your reasoning.

4. A number of different genetic variations can result in differences in sex differentiation. One example of this is a 46, XX male where crossing over between the X and Y chromosome results in the SRY gene being present on one of their X chromosomes.

a. Given what you have learned, would these individuals have functional androgen (testosterone) receptors and be able to respond to testosterone? Why or why not?

b. Would you predict that these individuals be able to produce levels of testosterone typical for biological males? Why or why not?

c. Looking at the range of testosterone levels from the graph above, predict an approximate serum testosterone level for a 46, XX SRY-positive male elite athlete. (This estimate need not be exact.) Explain your reasoning.

Once you have completed the questions, please get the Group 3: Part 2 Sheet from your instructor.

Group 3: Part 2

Actual serum testosterone level measurements from 15 different 46, XX SRY-positive males are below.

3.3 nmol/L	6.4 nmol/L	8.4 nmol/L
3.4 nmol/L	6.8 nmol/L	8.9 nmol/L
3.6 nmol/L	7.0 nmol/L	9.5 nmol/L
5.3 nmol/L	7.0 nmol/L	10.1 nmol/L
5.4 nmol/L	8.4 nmol/L	13.0 nmol/L

Use the data above to answer the following questions.

d. What is the range of serum testosterone levels in the individuals above?

e. What is the average of the serum testosterone levels measured for these individuals?

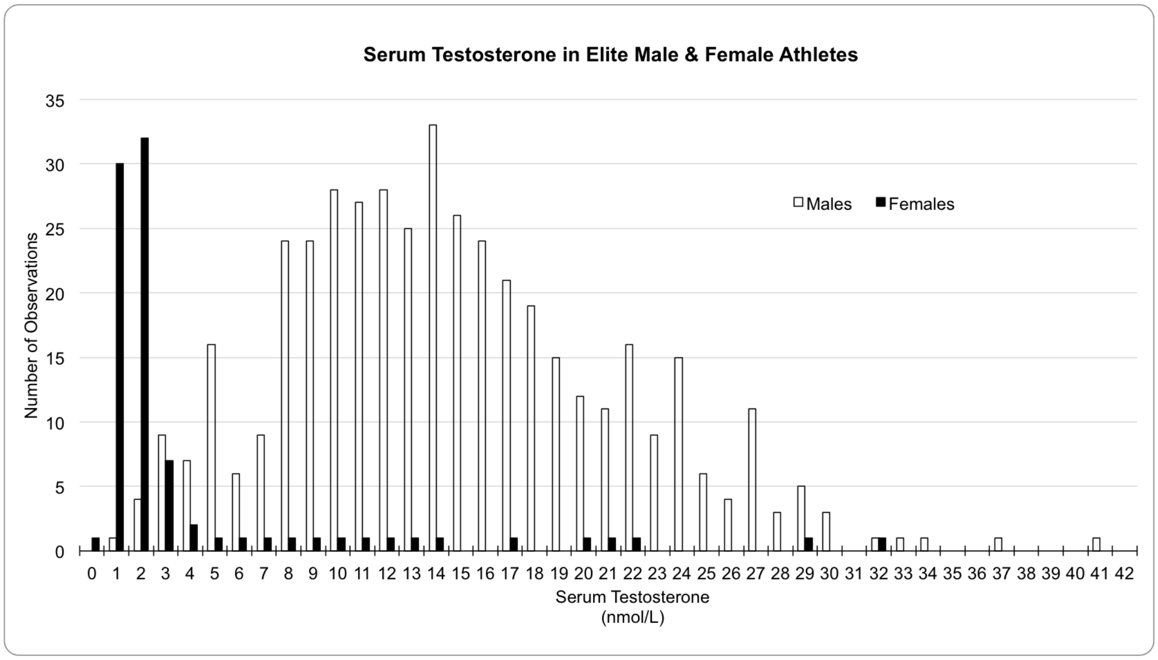
f. How far off was your prediction from the average? Was it within the range?

g. Given what you have learned, explain why 46, XX SRY-positive males have this level of testosterone.

References
Healy ML, *et al.* (2014) Endocrine profiles in 693 athletes in the postcompetition setting. *Clinical Endocrinology.* **81**, 294-305.

46, XX SRY-positive male testosterone levels from Akinsal et al (2017) Int Braz J Urol 43(4):770-775 and Wu et al (2014) BMC Urology 14:70.

Group 4: Part 1



Graph adapted from Healy *et al*, 2014

1. Based on the graph above, describe the trends you see in the testosterone levels of elite males and female athletes. (Who has more? What is the distribution?)

2. What is the range of testosterone levels measured in the male athletes displayed in the graph? What is the range of testosterone levels measured for the female athletes? Do the ranges overlap? (Remember to use labels.)

3. a. If an individual had a serum testosterone level of 9 nmol/L, would you be able to <u>definitively</u> determine the individual's biological sex? Why or why not?

b. What would your prediction of the individual's biological sex be? Explain your reasoning.

4. A number of different genetic variations can result in differences in sex differentiation. One example of this is 47, XXY males (a condition known as Klinefelter's Syndrome). These individuals have an SRY gene in their genome and phenotypically develop as male, but also have two X chromosomes.

a. Given what you have learned, would these individuals have functional androgen (testosterone) receptors and be able to respond to testosterone? Why or why not?

b. Given what you have learned, would you expect these individuals to have a Barr body in their cells? Why or why not?

c. Looking at the range of testosterone levels from the graph above, predict an approximate serum testosterone level for a 47, XXY male elite athlete. (This estimate need not be exact.) Explain your reasoning.

Once you have completed the questions above, please get the Group 4: Part 2 Sheet from your instructor.

Group 4: Part 2

Below are approximate testosterone levels measured from 11 different 47, XXY males. 1.4 nmol/L

1.4 nmol/L	7.3 nmol/L	12.5 nmol/L
4.5 nmol/L	8.0 nmol/L	14.9 nmol/L
5.2 nmol/L	7.6 nmol/L	25.3 nmol/L
6.2 nmol/L	11.5 nmol/L	12.5 nmol/L

Please use this data to answer the following questions.

d. What is the range of serum testosterone levels in the individuals above?

e. What is the average of the serum testosterone levels measured for these individuals?

f. How far off was your prediction from the average? Was it within the range?

g. How does the average and range of serum testosterone levels for 47, XXY males compare to those of typical males and females?

Note: Scientists actually do not understand why 47, XXY males have this range of testosterone given the functional SRY gene and the fact that X-inactivation leaves only one X chromosome active.

<u>References</u>

Healy ML, *et al.* (2014) Endocrine profiles in 693 athletes in the postcompetition setting. *Clinical Endocrinology.* **81**, 294-305.

Pacenza N, *et al.* (2012) Clinical presentation of Klinefelter's Syndrome: Differences according to age. *International Journal of Endocrinology*. **2012.**

<u>Optional Extension: Pre-lesson Homework with Readiness Assessment Quiz</u>
Prior to coming to class, complete the following activity. Watch the video "Test your knowledge of Sex Determination", https://www.hhmi.org/biointeractive/test-your-knowledge-sex-determination

The click and learn provides the fundamentals of genetic sex determination and compares the regulation of sex determination in different organisms.

MATERIALS

- HHMI BioInteractive "Test your knowledge of Sex Determination" click and learn. https://www.hhmi.org/biointeractive/test-your-knowledge-sex-determination
- Readiness assessment quiz.
- Computer, internet, projector.

Possible Quiz Questions:

1. A human who is XX and has male genitalia, would likely have:
   a. an additional X chromosome.
   b. a piece of the Y chromosome containing the SRY gene.
   c. any piece of the Y chromosome.

2. In order to have an XY mammal develop as a female:
   a. the X chromosome would have to be removed.
   b. the X and Y chromosomes would both have to be removed.
   c. the part of the Y chromosome containing the SRY gene would have to be removed.
   d. there is no way of making this mouse develop female.

3. Climate change experts suggest that temperatures are increasing, what could happen to reptiles?
   a. There will be more females than males.
   b. There will be more males than females.
   c. The numbers of males and females will become equal.
   d. Temperature does not impact reptile sex determination.

4. Individuals with Triple X syndrome have an extra X chromosome, thus an individual with this disorder will likely develop biologically as:
   a. male.
   b. female.
   c. both male and female.

5. A fruit fly has the genotype XXY. It will develop as:
   a. male.
   b. female.
   c. both male and female.
   d. neither male nor female.

Optional Extension: In-class Lesson with Quiz prior to activity

To provide you with more background knowledge prior to completing the in-class activity, your instructor will take you through the HHMI BioInteractive "Sex Verification in Athletes" click and learn Human Development section. You may follow along with PowerPoint handout. You may be asked to complete a quiz as well.

MATERIALS

- Click and Learn from HHMI activity: Sex Verification for Athletes, Human Development Section. https://www.hhmi.org/biointeractive/testing-athletes
- PowerPoint handout "Regulation of Sex Determination".
- Computer, internet and/or projector to watch/present click and learn and/or PowerPoint.
- In-class quiz.

Possible Quiz Questions:

1) What is testosterone?
   a. a disaccharide (carbohydrate)
   b. a steroid (lipid)
   c. a polypeptide (protein)
   d. a form of RNA (nucleic acid)

2) What is an autosome?
   a. A full 23 pairs of chromosomes
   b. The X chromosome
   c. The Y chromosome
   d. Any chromosome other than the X or Y chromosome

3) What is a Barr body?
   a. The active X chromosome of a pair
   b. The inactivated X chromosome of a pair
   c. A mutated X chromosome with an SRY gene
   d. A mutated Y chromosome missing the SRY gene

4) Where are Barr bodies typically found?
   a. in the nucleus
   b. in the nucleolus
   c. on a ribosome
   d. floating in the cytoplasm

5) Where are the precursors to the female reproductive system?
   a. Wolffian ducts
   b. Mullerian ducts
   c. The ureter
   d. Cloaca

6) What type of protein does the SRY gene encode?
   a. a transcription factor
   b. a hormone
   c. a cytoskeletal protein
   d. a cyclin

7) What determines whether and what type of secondary sex characteristics a person will develop?
   a. the external genital
   b. the presence of the SRY gene
   c. whether testosterone levels are over 5mol/L
   d. the relative amounts of estrogen and testosterone

8) What does the 45 refer to in “45, X”?
   a. the number of chromosomes the person has
   b. the chromosomal location of the person’s sex determining region
   c. the age of the person when their karyotype was completed
   d. the fact that the individual would typically be considered female

9) A person with a functional SRY gene will typically develop into a __________.
   a. male
   b. female

10) Androgen insensitivity will result in __________ that a person develops female characteristics.
   a. an increased chance
   b. a decreased chance