Development of the External Genitalia in Bovine Fetuses

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(Received for publication September 9, 1981)

Abstract. The external genitalia in bovine fetuses were studied macroscopically, with special reference to the formation of the labia and the sexual differentiation. The age of fetuses was expressed in crown-rump length (CRL). The female urogenital fold budding on either side of the genital tubercle gradually approached the tip of the genital tubercle by the 35-cm stage and then began to enclose it with the companion fold on the other side, thus forming the labium pudendi which is equivalent to the labium minus pudendi of the human. The genital swellings, which are known to become the labia majora in the human, did not take part in the formation of the labia. They completely disappeared at the sites cranial to the genital tubercle by the 10-cm stage. The sex difference in differentiation of the external genitalia was first observed at the 2.7-cm stage, at which time in the male, the urogenital orifice and the anogenital raphe appeared at the sites caudal to the genital tubercle; in the female at the 2.9-cm stage, both structures were still not observable. The anogenital distance became clearly longer in male than in female, after 3.6–3.9-cm stages.

The external genitalia of either sex are disparately differentiated from a common starting point. In early stages, the components of the external genitalia are formed on the caudoventral surface of the abdominal wall, as vaguely outlined elevations known as the genital tubercle, the genital swellings, and the cloacal folds. The cloacal folds presently separate into the urogenital folds and the anal folds when the urorectal septum subdivides the primitive cloaca.

In the male, the growth of the genital tubercle becomes markedly elongated to form the penis, the urogenital folds enclose the penis as the prepucce, and the genital swellings become enlarged to form the scrotum. In the female, particularly in the human, the genital tubercle becomes the clitoris, the urogenital folds become the labia minora, and the genital swellings develop into the labia majora [7].

Descriptions of the labial formation in domestic animals, though only very brief, can be encountered with some varied opinions. The first opinion is that the labia are formed by the genital swellings and the urogenital folds mostly disappear [5]. The second opinion is that the labia are formed by the urogenital folds and the genital swellings completely disappear (sheep) [1] or only vestigially remain (pig, rat) [3]. The third opinion is that the overall developmental pattern is similar to that in the human (pig) [6].
The present study was undertaken to know how and from what origins the labia of bovine fetuses are made up and also to clarify the critical period of the sexual differentiation of the external genitalia.

**Materials and Methods**

Sixty-five bovine fetuses (Holstein), ranging from 1.2 cm to 46 cm in crown-rump length (CRL), were used in this study. They were obtained from the Shibaura, Tama and Utsunomiya Meat Inspector's Stations. Fetuses less than 5 cm were fixed in toto in 10% formalin solution. Larger fetuses were treated as follows: A cranial half of the body was cut off at the diaphragmatic level and the liver and intestines were removed. Then, the body was put in 10% formalin solution. Only 33 cm and 46 cm fetuses were not fixed, immediately used for macroscopic observation of the external genitalia.

The anogenital distance was measured in all the fetuses used. After measurement, the formalin-fixed materials were transferred to Bouin's fluid and their external genitalia were examined.

The sex of fetuses larger than 3 cm in CRL was easily distinguishable by the outside view of the external genitalia, particularly by the size of the urogenital orifice and the presence of the anogenital raphe as described in the results. Smaller fetuses including a 3 cm one were subjected to histologic inspection of their gonads to know their sexes. The main criteria for determination of the male were the formation of seminiferous tubules and the appearance of Leydig cells in the gonad.

**Results**

Indifferent stage (<2 cm). In the midline between the umbilical cord and the tail, there was a small elevation known as the genital tubercle. On the top of the genital tubercle, an oval cloacal membrane, which was vaguely encircled by the cloacal folds, was situated. Somewhat cranial on either side, there was a vaguely outlined round elevation known as the genital swelling (Fig. 2).

Differentiated stage of the gonad (2~2.5 cm). At about this stage, the genital tubercle became vertically elongated and the cloacal folds separated into the urogenital folds and the anal folds. The genital tubercle had an undercut groove on its ventral surface (Fig. 5). The groove was surrounded by the urogenital folds throughout its entire length. The genital swellings were still round as vaguely outlined elevations.

Differentiated stage of external genitalia; 2.5~3 cm males. In this stage, the urogenital orifice and the anogenital raphe appeared at the sites caudal to the genital tubercle, the tip of which forwarded caudad. The small oval opening of the urogenital orifice became communicated with the urogenital sinus between both urogenital folds. Anteriorly, the orifice was continuous with the undercut groove on the ventral surface of the genital tubercle. The anogenital raphe appeared as a vague ridge-like thickening in the midline between the anogenital orifice and the anal folds (Fig. 4). The genital swelling became clearly elevated on either side of the genital tubercle. Two pairs of nipples appeared close to the midline cranial to the genital tubercle.

2.5~3 cm females. In this stage, the external genitalia differed from those in the above-described males by lacking the urogenital orifice and anogenital raphe (Fig. 5). Other features such as genital swellings, genital tubercle and nipples were almost the same as in the male.

3~4 cm males. The genital tubercle began to migrate along the abdominal wall towards the umbilicus. The male anogenital distance definitely increased from this stage (3.6~3.9 cm stages) (Figs. 1 and 6). The urogenital folds became elevated and they surrounded the basal region of the genital tubercle as the primary of the prepuce, where the hole-like urogenital orifice opened. The genital swellings became hemispherically elevated to form the scrotal pouches. The line of the anogenital raphe along the caudal direction of the urogenital
orifice was clearly marked by the persistence of a ridge-like thickening.

3–4-cm females. The slit-like urogenital orifice clearly appeared in the caudal direction of the genital tubercle. The urogenital folds became clearly elevated. There were little alterations about the genital swellings (Fig. 7).

4–5-cm males. The genital tubercle migrated further forward along the ventral body wall. According to this, the genital swellings, which had been located at first laterally to the genital tubercle, came to be located rather caudally to the tubercle (Fig. 8).

4–5-cm females. The genital swelling was still situated on either side of the genital tubercle, showing no migration and seemingly smaller than that of males of the similar age (Fig. 9).

Males larger than 5 cm. The genital tubercle reached the region close to the umbilicus. The genital swellings came to resemble the adult scrotum in such a way that both swellings fused with each other in the midline caudal to the genital tubercle (Fig. 10). Age by age, the urogenital groove became closed and the hole-like urogenital orifice moved to the tip of the genital tubercle. The nipples remained rudimentary on either side between the genital tubercle and the scrotum. At about 20-cm stage, the male external genitalia developed well, resembling the adult features.

5–6-cm females. The genital swellings came to be situated relatively cranial to the genital tubercle which did not so clearly move towards the umbilicus as the male. The genital swellings became smaller than those in the male, apparently undergoing degenerative changes (Fig. 11).

6–9-cm females. The genital swellings were conspicuously degenerated, indistinguishable in the region far cranial to the genital tubercle. Both genital swellings did not fuse with each other (Fig. 12).

Females larger than 9 cm. The genital swellings completely disappeared at the sites cranial to the genital tubercle by the 10-cm stage (Fig. 13). The urogenital folds were gradually elevated to surround the tip of the genital tubercle. Thereafter, they en-sheathed the genital tubercle to form the labia until the stage of about 45 cm when they seemingly resembled the adult labia (Figs. 14–17).

**Discussion**

The foregoing observations reveal that the way of formation of the labia in cattle is quite different from that in the human, pig, rat and dog. The genital swellings, which are known to develop into the labia majora in the human, do not take part in the formation of the labia in cattle. They completely disappear in females at about
10-cm stage, leading to the conclusion that the labia majora are absent in cattle. In early stages, the location of the genital swellings is approximately the same in both sexes. Then, in the female, the genital swellings come to be situated cranially to the genital tubercle, since the genital tubercle does not migrate along the abdominal wall towards the umbilicus. The genital swellings eventually come to be degenerated. In later stages, the urogenital folds, which are known to grow into the labia minora in the human, develop to be elevated on both sides of the genital tubercle and to extend up to its tip, finally enclosing it by the 33-cm stage. Thus, it is clear that in cattle the labia are formed only by the urogenital folds.

In the human, Spaulding [7] pointed out that the urogenital folds are differentiated into the labia minora as well as the preputium cridoidis, and that the genital swellings are differentiated into the labia majora. In the domestic animals, Martin [5] described that the urogenital folds almost disappear except for only a small part which forms the preputium cridoidis, and that the labia are formed by the genital swellings. In the pig and rat, Hannenberg [3] stated that the urogenital folds come to be the labia minora and that the genital swellings develop into the labia majora, but that the labia majora become inconspicuous immediately before or after birth. In the dog, Evans [2] described that whether the urogenital folds exactly form the labia is not clear, and that the genital swellings become differentiated into the labia. To be noted in particular is the finding presented by Böhm [1] in sheep in which the labia are formed by the urogenital folds and the genital swellings disappear in early fetal stages. Accordingly, our observations together with Böhm’s seem to imply that the labial formation only by the urogenital folds is a peculiar event in ruminants.

Sexual differentiation of the external genitalia could be distinguished from the appearance of the urogenital orifice and the anogenital raphe in the region caudal to the genital tubercle in a 2.7-cm male. But, such criteria were not observed even in a 2.9-cm female. By the time when the sexual differentiation can be detected from the outer view of the external genitalia, the gonads can be distinguishable whether male or female. Jost et al. [4] described that soon following the gonadal sexual differentiation, an alteration appears in the external genitalia as an increase in the anogenital distance in male, and that the distance begins to increase markedly from 3.4 cm to 3.6 cm (Days 48 to 49) until the genital tubercle reaches the umbilical region at 5.9 cm (Day 58). In the present study, it is found as shown in Figure 1 that the onset of increase in the anogenital distance in male occurs at a 3.6 cm stage just in harmony with the finding of Jost et al. [4]. Thus, the present study supports the view that the sexual differentiation of the external genitalia occurs at a stage so earlier than the stage when the anogenital distance begins to increase.

Acknowledgments. The authors are greatly indebted to Mr. M. Kuchii for his technical assistance. This work was supported in part by Grants-in-Aid (No. 448071 and No. 548067) from the Ministry of Education, Culture and Science in Japan. This paper is an abridgment of a part of the thesis submitted by Tomoo Inomata to the Graduate School of Azabu University in partial fulfilment of the requirements for the degree of D.V. Sci.

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要約

ウシ胎仔外生殖器の発達について、稲喰智夫・江口保樹・山本雄子・浅利昌男・鹿野幇（静岡大学獣医学部解剖学教室）、望月公子（東京大学農学部家畜解剖学教室）—ウシ胎仔外生殖器、とくに陰唇の形成と外生殖器の性分化について、実体顕微鏡を用いて観察した。陰唇の形成について——尿生殖ヒダは、体長（CRL）33 cm ぐらいの時期までに、生殖結節をほとんど彼うように発達して陰唇（ヒトでいう小陰唇に相当）となった。生殖腺起、すなわち、ヒトでいう大陰唇となるものは、体長の増加にともなって頭方に移動し、体長が10 cm になるころに生殖結節の頭方、尾骨部付近に退行消失した。よって、ウシに見られる陰唇は、生殖腺起に由来するものではなく、尿生殖ヒダに由来するものである。外生殖器の性分化について——雌で体長 2.7 cm の時期に、生殖結節の尾方、肛門との間に、わずかに隆起した会陰縫線と精円形の小さな尿生殖口が認められた。しかし、同じころの雄（体長 2.9 cm）では、会陰縫線や尿生殖口は認められなかった。よって、ウシ胎仔外生殖器が性分化する時期は、体長 2.7 cm 前後のところであるものと思われた。
Explanation of Figures

5. Genital swelling. 10. Tail (cut).

Fig. 2. (1.2 cm). The genital tubercle (6) is located in the midline between the umbilical cord (11) and the tail (9) as a small elevation. There is an oval cloacal membrane (4) on the top of the genital tubercle. It is vaguely encircled by the cloacal folds. Somewhat cranially on either side, the genital swelling (5) is situated as a vaguely outlined round elevation. ×17.1.

Fig. 3. (2.3 cm). The genital tubercle (6) become vertically elongated. Cranial to the genital tubercle, there are vaguely outlined elevations of the genital swellings (5). This material is identified as a male from the histologic view of its gonads, not from the outer view of the external genitalia. ×9.4.

Fig. 4. (2.7-cm male). A small oval opening of the urogenital orifice (14) and a vague ridge-like thickening of the anogenital raphe (1) can be noted at the sites caudal to the genital tubercle (6), suggesting that the material is a male, as supported by a histologic inspection of the gonad. The genital swellings (5) become clear and two pairs of nipples (7) appear. ×7.9.

Fig. 5. (2.9-cm female). The urogenital orifice and the anogenital raphe are not observable. Other features are almost the same as in the male in Figure 4. This material is identified as a female not only from this outer view of the external genitalia but also from the histologic feature of the gonad. ×7.9.

Fig. 6. (3.6-cm male). The urogenital folds surround the basal region of the genital tubercle to form the primary of the prepuce (8). The genital swelling (5) becomes hemispherically elevated and the anogenital raphe (1) becomes clear. ×7.7.

Fig. 7. (3.6-cm female). In the caudal direction of the genital tubercle (6), the slit-like urogenital orifice (14) is clearly opened. The urogenital fold (12) on either side of the orifice becomes clearly elevated. There are little alterations about the genital swellings (5). ×7.7.

Fig. 8. (4.5-cm male). The genital tubercle (6) migrated forward along the ventral body wall to be situated cranially to the genital swellings (5). According to this, the locational relationship of the genital swellings and the genital tubercle is reversed. ×6.5.

Fig. 9. (4.6-cm female). The external genitalia are similar to those of 3.6-cm female in Figure 7, except for an enlarged size of the genital tubercle (6). The genital swellings (5) are seemingly smaller than those of males of the similar age (cf. Fig. 8). ×6.6.

Fig. 10. (6.1-cm male). The genital tubercle (6) reached the region close to the umbilicus. In the midline caudal to the genital tubercle, both genital swellings (5) fuse with each other. ×4.5.

Fig. 11. (5.9-cm female). The genital swellings (5) are situated cranially to the genital tubercle (6). They become degenerated and smaller than those of the male (cf. Figure 10). ×4.9.

Fig. 12. (7.5-cm female). The genital swellings (5) are markedly degenerated, not fusing in the midline as in males. ×3.2.

Fig. 13. (10.0-cm female). The genital swellings completely disappear in the region cranial to the genital tubercle (6). The labia majora, which come to be formed by the genital swellings in the human, are absent in cattle. ×2.4.

Fig. 14. (10.0-cm female). The left-caudal view of the material shown in Figure 13. The genital tubercle (6) is still situated close to the tail (10). The genital tubercle and the urogenital folds (12) show little alterations from the 3.6-cm stage (Figure 7) to this stage. ×3.9.

Fig. 15. (19.0-cm female). The genital tubercle (6) is largely surrounded by the urogenital folds (12). ×1.2.

Fig. 16. (33.0-cm female). The urogenital folds (12) become developed to ensheath the genital tubercle (6). ×1.1.

Fig. 17. (46.0-cm female). The urogenital folds (12) completely engulf the genital tubercle to become the labia. ×0.7.