Sexual Differences in Closure of the Human Palatal Shelves

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Clinical data have shown that sexual differences in the incidence and severity of cleft palate types may stem from a sexual dimorphism in the timing of human palatal shelf closure (*7*). These data generally indicated that female embryos show a greater incidence and severity of palatal clefting than do male embryos of the same age. Based on a hypothetical model of human palatal closure, such sexual differences in the clinical data were explained on the assumption that the secondary palate of the female embryo closes at a slower rate than that of the male embryo of the same age. The suggested female time lag further indicates that since the female secondary palate is open for a longer period of time than is that of the male, the female embryo may be susceptible to teratogenic disruption of normal palatal closure for a greater period of time.

This stimulating developmental model suggests differential rates of palatal closure for the two sexes, and it correlates well with the cleft palate incidence studies of Knox and Braithwaite (*5*), Fogh-Andersen (*2*), and Mazaheri (*6*). The pooled 619 clinical cases of these investigators shows a 1.5/1 female to male ratio for palatal clefts of all types, i.e., complete, partial. More specifically, these collective data indicated a 2.0/1 female to male ratio for complete clefting of the secondary palate and a 1.2/1 ratio for clefts of the soft palate.

The present study is a follow-up to the report by Meskin, Pruzansky, and Gullen (*7*), and is based on two primary questions: (1) Is there an identifiable sex indicator for human embryos during the times of palatal closure? (2) Are there any discernible trends in the relationship between the sex of the human embryo and early stages of its palatal development?

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Materials and Methods

The sample for this study consisted of 46 human embryos from The University of Michigan Embryolcgy Research Collection, Department of Anatomy. This sample ranged in size from 18 through 55 millimeters crown-rump length and in age from approximately 7 through 10 weeks. The composition of the sample was necessarily limited to embryos that represented the secondary palate before, during, and immediately after palatal closure. Twenty-six embryos in the sample were identified as male; 20 were females.

Light microscopy was used to evaluate both the stage of palatal development as well as the sex of the embryo. Each embryo was routinely fixed in 10% neutral formalin, paraffin embedded, and sectioned. Sections were made frontally through the embryo's head and transversely through the trunk. Serially-mounted sections were alternately stained with combinations of aldehyde-fuchsin, hematoxylin and eosin, or a Masson connective tissue stain.

In order to eliminate, or at least minimize, an observer bias, all embryos were first evaluated for palatal development and then examined to identify their sex. Only after the completion of both examinations were the two types of data correlated. The criteria and procedures for both examinations are as follows.

A. Evaluation of Palatal Development. The palatal region of each embryo was examined at five arbitrary depths or levels to identify the anteroposterior sequence of palatal closure: (Level 1) the junction of the primary and secondary palates; (Level II) the location of Jacobson's organ; (Level III) the location of either the descending palatine nerves or the posterior limits of the dental ridge; (Level IV) the loss of septopalatine contact; and (Level V) a mid-point along the developing soft palate. Levels IV and V were especially appropriate for embryos of 30 millimeters crown-rump length or older. The positions of the shelves were assigned arbitrary developmental points and rated on a seven-point scale: *1,* no shelf; *3,* shelves vertical at the sides of the tongue; *5,* horizontal and open shelves; and *7,* horizontal and closed shelves (intermediate stages of closure, *2, 4,* and *6,* as well as asymmetries were also recorded).

B. Identification of the Embryo's Sex. According to the classic study by Gillman (*4*) on the development of the gonads in man, the differentiation of the human gonad into a testis can typically be identified in embryos of 14 to 16 millimeters crown-rump length. (At this age the palatal shelves begin to arise from the walls of the oronasal cavity and are directed vertically along the sides of the tongue.) Differentiation of the testis is much more evident than is that of the ovary. The key criteria used by Gillman and by us to identify a testis were a) a relative prominence of deeper staining seminiferous

tubules or cords separated by spindle-shaped mesenchymal cells, b) a decrease in the number of primordial cells within the cellular zone of the young testis, and c) a thickened tunica albuginea. In general, the presence of the seminiferous tubule system was the most important single sex criterion to use.

In general, and according to Gillman, the young ovary is identified by the fact that it is not a testis. Identification of the ovary, as it emerges from the gonadal indifferent stage at 17 millimeters crown-rump length, is made chiefly on negative morphologic features. That is, the absence of a testis and presence of a young ovary can be established by a relative void of seminiferous cords and by a relatively thin outer covering for the organ. Unlike the male, however, in the female embryos examined there was a persistence of a large number of primitive sex cells in the outer cellular zone of the differentiating ovary.

Results

Tables 1 and 2 indicate that the palatal shelves for the two sexes change from an early vertical (position 3) to horizontal (position 5) orientation at different developmental ages, determined by crown-rump length. In the sample of 26 male embryos, the three anterior-most levels of the palatine shelves were in a horizontal position in 65% of the cases at 25 millimeters crown-rump length or approximately the seventh week. This change in shelf position occurred later in the female group (Table 2). Prior to the middle of the eighth week or 30 millimeters crown-rump length, 60% of the twenty female embryos had their three anterior depths of the palate in a vertical position. Generally, in both male and female embryos, the patterns of shelf position in the future soft palate regions (depths IV and V) were not as consistent as those observed for the anterior palatal regions.

This observation about the differences in palatal closure in male and female embryos might also be shown on the basis of percentage completion of the entire secondary palate. As shown in Tables 1 and 2, this percentage was determined by assigning seven developmental points to the last possible stage of palatal closure in each of the five depths of the palate. Thus, if the three anterior depths were completely closed, that region would have 21 developmental points and be 100% completed. Regional percentage completions data were thus determined. In general, the three anterior regions of the palate in the female embryos at 30 millimeters crown-rump length were less than 50% completed. In the males, the 50% level of completion for the same palatal regions appeared typically at the 25 millimeter crown-rump length stage.

Observations in this study also demonstrated the anteroposterior gradient of shelf closure as well as supero-inferior gradient of shelf consolidation. The two sexes showed no difference in these features.

TABLE 1. Regional closure of the male embryonic palate.

embryo number[1]	*CRL (mm)*	*anterior palate depths*			*% develop.*	*posterior palate depths*		*% develop.*	*total % develop.*[2]
		I	*II*	*III*		*IV*	*V*		
EH 358	19	3	3	3	43	3	1	29	13/35 37
EH 231	21	3	3	3	43	3	1	29	13/35 37
EH 102	21	3	3	3	43	3	1	29	13/35 37
EH 380	22	3	3	3	43	3	3	43	15/35 43
EH 739	22	3	3	3	43	1	1	14	11/35 31
EH 419	23	3	3	3	43	2	1	21	12/35 34
EH 147	23	3	3	3	43	3	2	36	14/35 40
EH 164	25	5	5	5	71	5	5	71	25/25 71
EH 938	26	5	5	5	71	5	1	43	21/35 60
EH 589	26	5	3	3	52	3	3	43	17/35 49
EH 892	27	3	3	3	43	3	1	29	13/35 37
EH 240	28	5	5	5	71	4	1	36	20/35 57
EH 888	29	5	5	5	71	5	5	71	25/35 71
EH 015	30	6	6	6	86	2	2	29	22/35 62
EH 377	31	5	5	5	71	4	2	43	21/35 60
EH 217	33	6	6	6	86	5	1	43	24/35 69
EH 946	34	6	6	6	86	6	1	50	25/35 71
EH 785	35	5	5	4	67	4	1	36	19/35 54
EH 523	36	6	6	6	86	5	1	43	24/35 69
EH 829	37	7	7	7	100	6	5	79	32/35 91
EH 621	38	7	7	7	100	6	6	86	33/35 94
EH 909	40	6	6	6	86	6	5	79	29/35 83
EH 593	41	7	7	7	100	7	7	100	35/35 100
EH 356	45	7	7	7	100	2	1	21	24/35 69
EH 610	48	6	6	6	86	6	6	86	30/35 86
EH 370	54	7	7	7	100	7	7	100	35/35 100

[1] Specimens from The University of Michigan Embryology Research Collection, Department of Anatomy.

[2] Percentage development is determined by dividing the number of developmental points assigned to the region by the total possible points (anterior, 21; posterior, 14; total, 35). Developmental points: 1, no shelves; 3, vertical shelves; 5, horizontal and open shelves; 7, horizontal and closed shelves; and 2, 4, 6, intermediate stages.

Discussion

Observations from this study suggest that the secondary palate of the male human embryo is more advanced in closure than is the female palate during the critical periods of palate formation. Trends indicate that the critical period of palatal closure for the male embryo is the

TABLE 2. Regional closure of the female embryonic palate.

embryo number[1]	*CRL (mm)*	*anterior palate depths*			*% develop.*	*posterior palate depths*		*% develop.*	*total % develop.*[2]
		I	*II*	*III*		*IV*	*V*		
EH 650	18	2	2	1	24	1	1	14	7/35 20
EH 619	18	2	2	1	24	1	1	14	7/35 20
EH 933	20	3	3	1	33	1	1	14	9/35 26
EH 600	20	5	5	3	62	3	3	43	19/35 54
EH 253	20	3	3	3	43	1	1	14	11/35 31
EH 420	20	3	1	1	24	1	1	14	7/35 20
EH 592	21	3	3	3	43	1	1	14	11/35 31
EH 598	22	3	3	3	43	3	1	29	13/35 37
EH 880	24	3	3	3	43	1	1	14	11/35 31
EH 591	25	3	3	3	43	3	1	29	13/35 37
EH 500	25	3	3	3	43	2	1	21	12/35 34
EH 840	28	3	3	3	43	2	1	21	12/35 34
EH 512	29	3	3	1	33	1	1	14	9/35 26
EH 948	30	6	6	6	86	6	5	79	29/35 83
EH 678	30	6	5	4	71	3	2	36	20/35 57
EH 479	36	6	6	6	86	5	2	50	25/35 71
EH 746	37	6	6	6	86	1	1	14	20/35 57
EH 609	40	6	6	6	86	6	3	64	27/35 77
EH 018	45	7	7	7	100	7	7	100	35/35 100
EH 784	55	7	7	7	100	7	7	100	35/35 100

[1] Specimens from The University of Michigan Embryology Research Collection Department of Anatomy.

[2] Percentage development is determined by dividing the number of developmental, points assigned to the region by the total possible points (anterior, 21; posterior, 14; total, 35). Developmental points: 1, no shelves; 3, vertical shelves; 5, horizontal and open shelves; 7, horizontal and closed shelves; and 2, 4, 6, intermediate stages.

seventh week (25 millimeters crown-rump length) as compared to the mid-eighth week for the female embryo.

In a study of the timing of palatal closure without specific reference to sex, Fulton (*3*) has shown that the critical period of palatal closure is near 30 millimeters crown-rump length with a range from 29 to 33 millimeters. The present study supports the findings from the earlier study in that Fulton has indicated that all embryos in his sample below 29 millimeters crown-rump length had vertically oriented palatal processes, whereas the present study shows that 30 millimeters may be specific only for the female embryo with 25 millimeters crown-rump length being the critical time for the male in the shifting of the palatal shelves from vertical to horizontal. No sex differences were noted for either the directional gradient or possible mechanisms (*1*) of palatal shelf closure.

It is especially important to note that the findings of this study are discussed appropriately as suggestions or possible group trends. Discussion of this type is warranted by a combination of the cross-sectional sampling technique imposed by the use of human prenatal specimens and the limited number of embryos available for histologic observations. Until this study can be supplemented by further investigations, the question will remain open on how representative our sample is of the universe relative to human palatal closure *in utero*.

If, however, the trends demonstrated by both Fulton and by the present study are indeed the typical patterns of human palatal closure relative to timing and sex, the hypothetical model of human palatal closure suggested by Meskin, Pruzansky, and Gullen (7), may, in fact, be a realistic one. That is, if the susceptible period of palatal development can be defined as terminating with the horizontal positioning of the shelf over the tongue, then the human female embryo, as compared to the male of the same age, not only shows a time lag in palatal closure but has a longer period of teratogen susceptibility.

Summary

This study was primarily concerned with the possible relationship between human palatal closure and sex of the embryo. Forty-six human embryos (26 males, 20 females) representing stages of palatal closure from the initial onset of the palatal shelves to the completion of the secondary palate were studied. Only specimens free from gross body defects were included in the sample. Light microscopy of the histologically-prepared specimens was used to evaluate the stages of palatal closure as well as to identify the sex of each embryo using established criteria that are specific to either the newly differentiated testis or ovary. Based on the cross-sectional observations of this study, it is suggested that the horizontal positioning of the male secondary palatine shelves occurs somewhat earlier than in the female. This general pattern of a relative lag in female palatal closure, if supported by future studies, implies a longer period of open palatine shelves for the female. As a corollary, the female embryo might have a longer period of teratogen susceptibility. This possible period of lengthened susceptibility correlates well with a reported hypothetical model of human palatal closure designed to offer an explanation for the greater incidence and severity of palatal clefting shown for the female in epidemiologic treatment of clinical data.

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