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Study of Fetal Sex Determination Based on External Genitalia and Gonadal Differentiation in the Water Buffaloes of Iran

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Abstract: This study was Performed on 102 embryos and fetuses that collected from Ahvaz slaughter house. The specimens was divided in three group, on the basis of external genitalia differentiation and Crown Rump Length (CRL), as groups 1, 2 and 3. The embryos of group 1 (No. 63, CRL-10-83 cm) showed completely developed external genitalia of male and female, so there was no doubts in the recognition of their sexes. The embryos of group 2 (No. 19, CRL-4-10 cm) also showed clear sex on the basis of genital tubercle (clitoris in the female and penis in the male embryos) and genital swellings (lips of vulva in the females and scrotom in the males). The tip of genital tubercle of females embryos have showed dorso-caudally and of males ventro-cranially directions, respectively. In the embryos of group 3 (No. 20, CRL = 0.38-4 cm). The determination of sex was not possible by help of external genitalia. So, the gonads of these embryos, were removed from abdominal cavity and after histological preparations, stained by H and E and PAS methods. On the basis of histological studies, sex differentiation of gonads to testis and ovary was recognized in embryos with CRL = 2.1 cm and CRL = 2.3 cm, respectively. Also the gonads of embryos lesser than CRL = 2.1 cm, recognized as undifferentiated gonads. These findings indicated that, sex differentiation in the level of gonads start sooner than external genitalia and also the differentiation of sex in male embryos has priority to female embryos. In addition, the embryos show an undifferentiated stage, that is impossible to recognize the sex of embryos neither on the basis of external genital nor gonadal histological structure.

Key words: Sex,differentiation, gonad, external genitalia, water buffalo

INTRODUCTION

Mammalian sex determination proceeds in three distinct phases. In the first stage, genetic sex is determined at the time of fertilization by the chromosomal complement of the fertilizing spermatozoon. Later, during embryonic development, this genetic information is translated in to gonadal sex that determines the growth of either a testis or ovary from a bipotential early indifferent gonad.

The third stage which is the phenotypic sex determination, begins in fetal or early post-natal life and continues through puberty, a period in which endocrine products of the gonads direct the differentiation of the accessory sex ducts and external genitalia (Loffler and koopman, 2003). External and gonadal sex differentiation has been studied in human and domestic animals with different results.

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In male human fetus, at 7th week of fetal life, under the influence of Y chromosome, primary sex cords containing proliferated coelomic epithelium and primordial germ cells continue their proliferation and form medullary (testicular) cords. Synchronously a thick fibrous layer called tunica albuginea forms due to proliferation of mesenchyme. The female gonads differentiate later than male.

Because of the absence of Y chromosome, the primary sex cords degenerate and coelomic epithelium proliferates and form cortical or secondary sex cords that are characteristic of early female gonad (Sadler, 2004).

Sex differentiation has been studied in human (Baker and Scrimgeour, 1980), swine (Inomata *et al.*, 1993), dog (Evans, 1979), bovine (Erichson, 1966), horse (Sakai, 1955) and goat (Harshan *et al.*, 1994; Banankhojasteh *et al.*, 2006). However, only few studies on ovarian differentiation in water buffalo fetuses was reported (Ghannam and Deeb, 1967, 1969). In many parts of the world, water buffaloes (*Bubalus bubalis*) are raised for production of milk, meat, hide and down. Water buffaloes have high adaptability and can live in different climates. During recent decades, universal concentration on water buffaloes has increased specially in Asian countries and new initiatives have been starting for development of related industries. Therefore, conduction of basic and applied research regarding various aspects of water buffaloes seems necessary.

Because there is no comprehensive study on external and gonadal sex differentiation in fetus and in view of the fact that studies on various aspects of Iranian native water buffaloes including fetal development are necessary, the present study was conducted to determine sex differentiation in water buffalo fetuses.

MATERIALS AND METHODS

During 2006, one hundred-two pregnant uteri in all stages of pregnancy were collected from Ahvaz slaughter house, in southern Iran. Fetuses were expelled after splitting of uterus and separating of fetal membranes.

Crown-Rump Lengths (CRL) and Crown-Vertebral-Rump Lengths (CVRL) of fetuses were measured so that the minimum CRL was 0.38 cm and maximum CRL was 83 cm also on the basis of CVRL, the minimum age estimation was less than 30 days and the maximum was 267.04 days (Abdel Raof and Elnaggar, 1968).

The fetuses were divided in three groups, on the basis of external genitalia differentiation and CRL, as groups 1, 2 and 3.

The embryos of group 1 (No. 63, CRL = 10-83 cm) showed completely developed external genitalia of male and female, so there was no doubts in the recognition of their sex.

The embryos of group 2 (No. 19, CRL = 4-10 cm) were also examined macroscopically to study the growth of external genitalia. In male fetuses, presence of long anogenital raphe between anus and genital tubercle (penis), existence of urogenital orifice and ventro-cranially direction of penis and in female fetuses, absence of anogenital raphe and urogenital orifice, growth of vulvar labia and dorso-caudally direction of clitoris were considered as the criteria for sexual differentiation. In fetuses of group 3 (No. 20, CRL = 0.38-4 cm).

Differentiation of sex was not possible by help of external genitalia characteristics, so cranial half of the body at diaphragmatic level was cut away. Then, serial sections, 6-micron thick, were prepared from the rest of the body and after staining with hematoxylin and eosin (H and E) and Periodic Acid Schiff (PAS), their gonadal differentiation were studied microscopically. Formation of tunica albuginea and medullary (testicular) cords in testis and formation of cortical and medullary region in ovary were considered as criteria for gonadal differentiation.

RESULTS

We found that water buffalo fetuses were macroscopically sexually differentiated externally after 4 cm CRL (CVRL = 6 cm, age = 56 days).

Male fetuses had long anogenital raphe between the anus and base of genital tubercle and also urogenital orifice. The tip of genital tubercle showed ventro-cranial direction too. But female fetuses present formation of vulvar labia, absence of urogenital orifice and anogenital raphe, short distance between anus and base of genital tubercle, as well as the tip of clitoris directed dorso-caudally (Fig. 1).

Microscopic studies of undifferentiated externally fetuses (less than 4 cm CRL) showed that gonads were not differentiated before 2.1 cm CRL (age = 45.74 days) (Fig. 2).



Fig. 1: Differentiated embryo to female sex, CRL = 1.6, CL = clitoris, the tip of clitoris is directed dorso-caudally

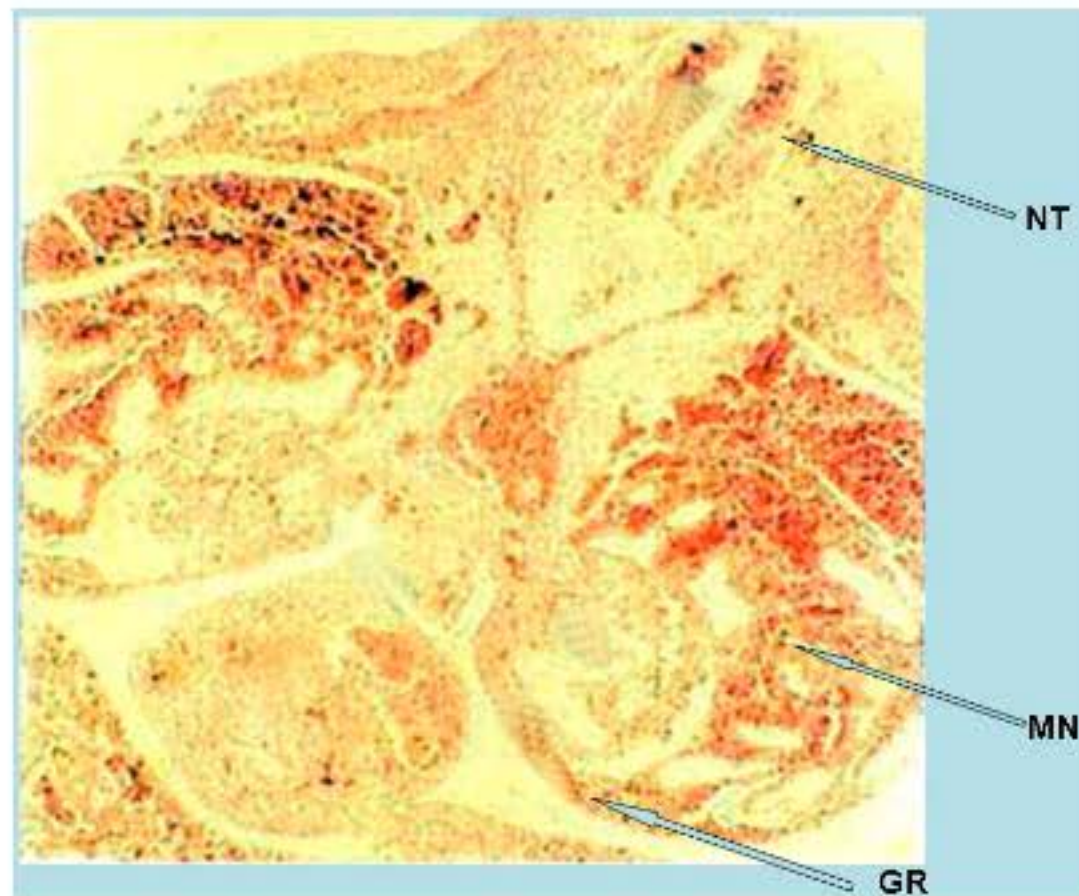


Fig. 2: Photomicrograph of undifferentiated gonad embryo of water buffalo, CRL = 0.77 cm. NT = Nural tube, MN = Mesonephros, GR = Genital ridge

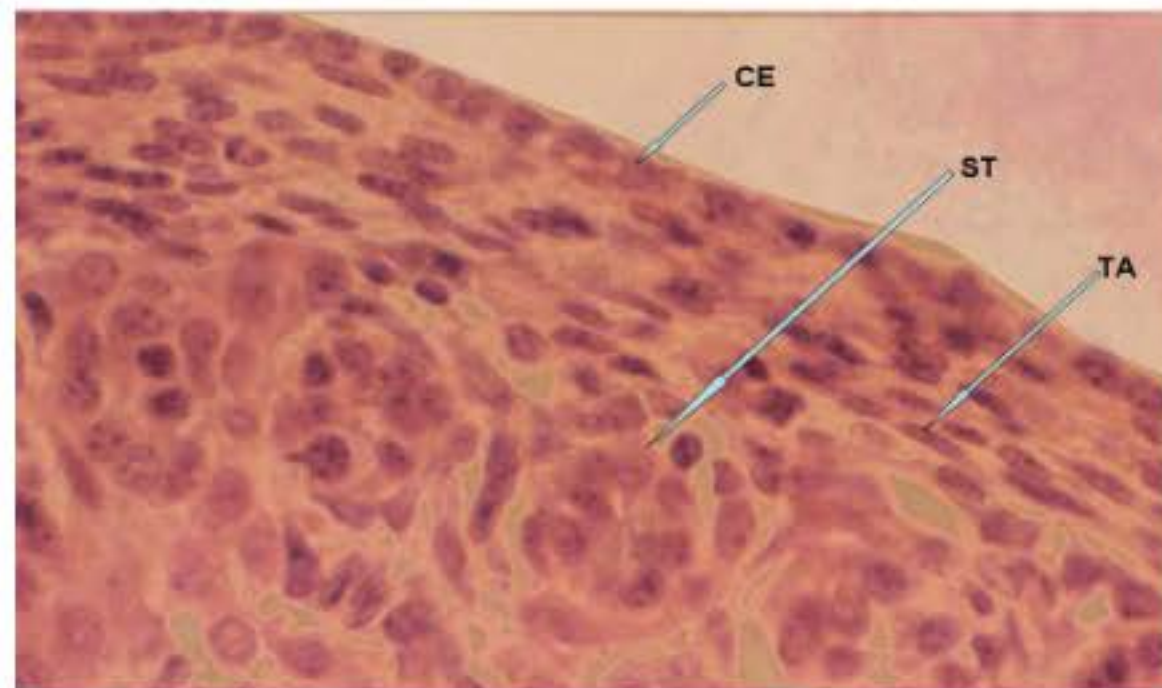


Fig. 3: Photomicrograph of testis of water buffalo embryo, CRL = 3.5 cm, PAS×100, CE = Celomic Epithelium, TA = Tunica Albuginea, ST = Seminiferous Tubule

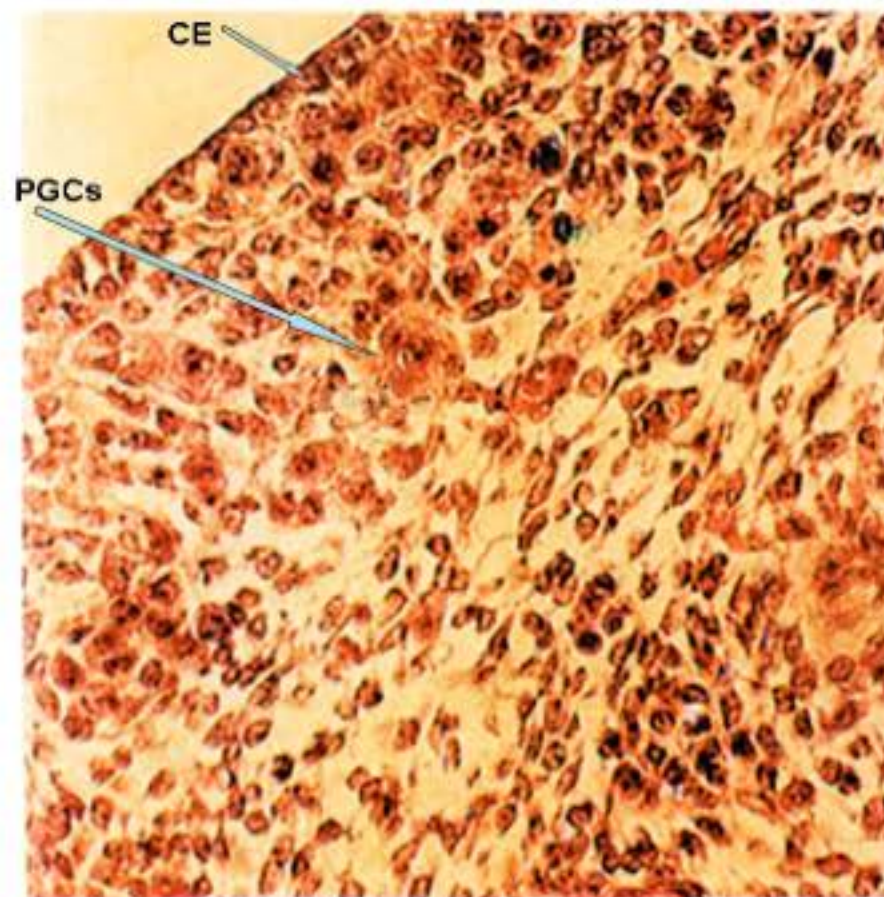


Fig. 4: Photomicrograph of ovary of water buffalo embryo, CRL = 3.8 cm, PAS = 3.5 cm
CE = Celomic Epithelium, PGCs = Primordial Germ Cells

So, at this time, in testis, tunica albuginea were formed under the surface of epithelium and medullary (testicular) cords were well-established (Fig. 3).

Differentiation of ovary was seen at 2.3 cm CRL (age = 47.1 days) with formation of clear cortex containing cortical (secondary) cords and medulla having blood vessels and connective tissue (Fig. 4).

DISCUSSION

The finding of the present study showed that recognition of testis in male water buffalo fetuses, by formation of the tunica albuginea and medullary cords are distinctive at 2.1 cm CRL (age of 45.74),

by formation of the tunica albuginea and medullary cords. However, tunica albuginea organize in swine at 30 days of fetal life, sheep at 35, cow at 45 (Noden and Delahunta, 1985) and goat at 36 days (Banankhojasteh *et al.*, 2006).

Some researchers reported that testicular differentiation in buffalo occur in 20-21 mm CRL (Ghannam and Deeb, 1967, 1969), that is comparable with our results. In this study the first signs of ovarian differentiation in female water buffalo fetuses was distinguishable at 2.3 CRL (age of 47.1 days) by organized cortical and medullary regions and absence of tunica albuginea.

There are many reports about ovarian differentiation in female fetuses of domestic animals, goat 38-40 mm, age of 40.5 days (Banankhojasteh *et al.*, 2006; Harshan *et al.*, 1994), sheep, at days 38 (Sawyer, 2002), cow at 25-35 mm (Bascom, 1923; Inomata *et al.*, 1982). Nonetheless, Sakai (1955) observed the cords only at 150 mm CRL. In swine, the external sex differentiation has been observed at 25-30 mm CRL (Inomata *et al.*, 1993).

Our finding indicated that sex determination in the level of gonads start sooner than external genitalia. Also in the level of gonads, male sex differentiation is occurred sooner than female gonads. This priority can be due to affections of Y chromosome. In addition, the embryos showed an undifferentiated stage, that recognition of sex was impossible neither on external genitalia nor gonadal histological structure.

Finally, comparison of various species show that many aspects of sex differentiation in water buffalo, are similar to other domestic animals specially cattle.

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