# Study Of Fetal Sex Determination Based On External Genitalia And Gonadal Differentiation In Water Buffalos

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**Citation**

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**Abstract**

This study was performed on 102 embryos and fetuses that were 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-10-83cm) 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-4-10cm) 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 scrotum in the males). The tip of genial 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 & E and PAS methods. On the basis of histological studies, the sex differentiation of gonads to testis and ovary were recognized in embryos with CRL=2.1 cm, and CRLI<2.3 cm respectively. The gonads of embryos with CRL<2.1 cm were recognized as undifferentiated. These findings indicated that the sex differentiation starts earlier in the gonads compare to external genitalia but the differentiation of gonad and external genitalia of male has priority to female embryos.
The sex of embryos were not recognized in undifferentiated gonad neither by external genitalia nor histological structure.

## 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.

In male human fetus, at $7^{th}$ 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 (Erickson, 1966), horse (Sakai, 1955) and goat (Harshan et al., 1994 and banankhojasteh et al., 2006). However, only few studies on ovarian differentiation in water buffalo fetuses was reported (Ghannam and Deeb,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 in creased especially 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 fetus.

## MATERIAL 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 83cm. also on the basis of CVRL, the minimum age estimation was less than 30 days and the maximum was 267.04 days (Abdel Raof, et al., 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= 10-83cm) 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= 4-10cm) 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 labia of vulva and dorso-caudally direction of clitoris were considered as the criteria for sexual differentiation. In fetuses of group 3(No. 20, CRL=0.38-4cm).

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&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 4cm CRL (CVRL=6cm , age= 56 days).

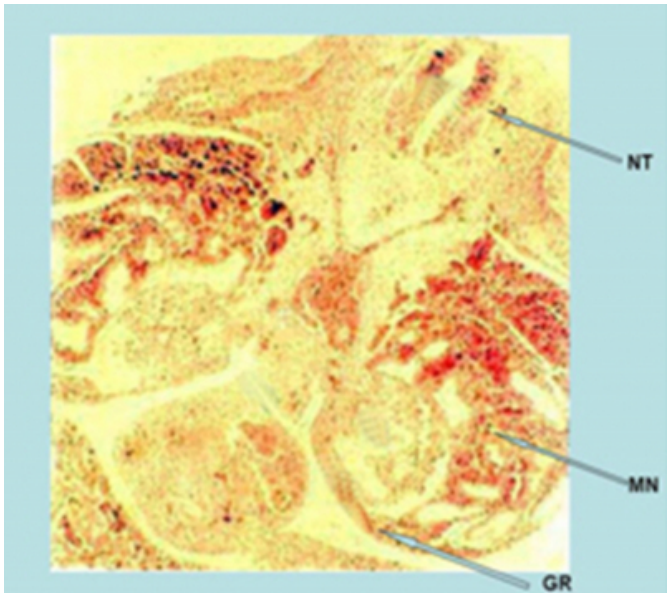
Male fetuses had long anogenital raphe between the anus and base of genital tubercle and also urogenital orifice. The tip of genital tubercle showed ventro-craniad direction too. But female fetuses present formation of vulva labia, absence of urogenital orifice and anogenital raphe, short distance between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

### Figure 1

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

**Figure 2**

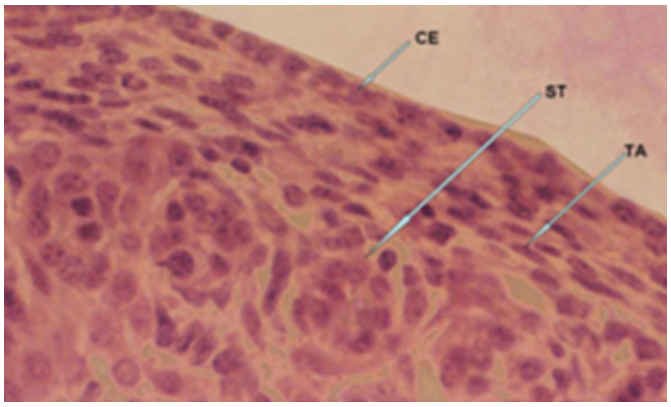
Figure 2: The undifferentiated gonad of buffalo embryo with CRL=0.77 cm( H&E, X 3.2). NT=Neural tube, MN=Mesonephrose , GR=Genital ridge .




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

**Figure 3**

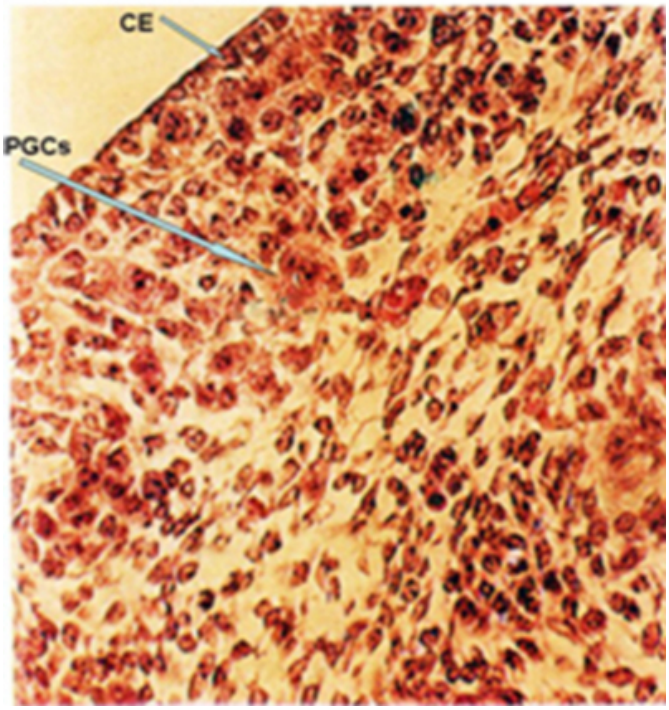
Figure 3: The testis of water buffalo embryo with CRL= 3.5 cm (PAS, X100). CE =Celomic epithelium , TA= Tunica albuginea, ST= Seminiferous tubule .




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).

**Figure 4**

Figure 4: The ovary of water buffalo embryo with CRL = 3.8 cm (H&E, X40). CE = Celomic epithelium, PGCs = Primordial germ cells .




## DISCUSSION

The finding of the present study showed that recognition of testis in male water buffalo fetuses, is 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 occurs in 20-21mm CRL (Ghannam and Deeb, 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 38-40mm, age of 40.5 days (bannankhojasteh,et al., 2006, Harshan. Et al., 1994), sheep, at days 38 (sawyer,2002), cow at 25-35 mm (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 25-30mm 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 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 histological structure.

Finally, comparison of results with other reports about various species showed that many aspects of sex differentiation in water buffalo, is similar to other domestic animals specially cattle.

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