Early determination of fetal sex in goat a comparison between real time PCR and ultrasonography

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Abstract. The point of the current study is to assess the productivity of the real time PCR and ultrasound techniques in early determination of fetal sex in Iraqi singleton pregnant goats. Our investigation has been led in Iraq, Al-Diwanya city from 10/8/2020 – 15/1/2021. The examination incorporates 45 singleton pregnant Iraqi goats, which initially inspected by ultrasound to affirm pregnancy and to decide the fetal sex depending on the restriction of the genital tubercle of the goat fetuses, after that, blood specimens had been gathered from the jugular vein of all examined does to detect fetal sex by discovery of AMLX and SRY genes in the circling cells free fetal DNA (ccffDNA) in these maternal blood specimens by utilizing real time PCR. Our outcomes showed an exceptionally high level of accuracy in real time PCR in contrast with the ultrasound strategy. The outcomes were affirmed by the true fetal sex after parturition in the inspected does. The complete symptomatic rate were 51.11% (23/45) and 97.78% (44/45) for ultrasound and PCR strategies separately. The exactness level of genuine analyzed female and male caprine kidding were 58.33% (7/12), 48.48% (16/33), and 100% (12/12), 96.97% (32/33) for ultrasound and real time PCR techniques separately. While the exactness rates of the two techniques utilized in this investigation for early caprine fetal sexing in respect to early pregnancies periods analyzed uncovered 100% (13/13), 96.3% (26/27), 100% (5/5), and 61.54% (8/13), 40.74% (11/27), 80% (4/5) in early pregnancy periods (58-62, 63-67, 68-73) days for real time PCR and ultrasound strategies individually. In conclusion our outcomes revealed a huge predominant exactness and productivity in fetal sexing in Iraqi singleton pregnant does in early development periods, with very high accuracy in real time PCR in compare to ultrasound techniques.

1. Introduction
Goats are viewed as a wellspring of milk and meat for human consumption, subsequently it's essential to expand their population. Perhaps the most strategy to build goats populaces is by utilization of the distinctive helped regenerative advances, and the productive administration procedures, for example, pregnancy finding strategies particularly the clinical once in which the ultrasonography is the principle of them, yet with number of inconveniences including expecting to a decent ability and time consumer. Exact fetal sex determination in domesticated species has huge financial and exploratory implications, particularly in the animal industry[1]. The ability to predict fetal sex in a species could be useful in making management decisions, such as sex selection in rearing projects, and lowering the
cost of offspring testing[2]. This further develop us to foster another unmistakable, quick and modest techniques for pregnancy determination. A few reproducers leaning toward a unique fetal sex. As of late, the molecular strategies particularly PCR had been applied as an elective technique for fetal sexing other than ultrasonography with high exactness rates in a few domestic animal groups like sheep [3], camel[4], goat[5], ovine and[6] just as in human[7]. Anyhow the productivity, these molecular techniques are quick, fairly cheap, need no expert people to apply them, and simple to gather tests. Yet, the utilizing of these molecular procedures along with ultrasonography in a few administration conditions and projects particularly with the other helped regenerative innovations like planned impregnation, estrous synchronization, and the powerful strategies for estrous recognition, these will be vital devices to foster the reproducing industry including caprine rearing. Then again, creating of such molecular procedures, particularly in the early growth period will assist with finding high number of teratogenic and hereditary issues during pregnancy and ensuing treatment of them, or possibly to prompt early terminations in the untreated cases. Utilizing of molecular PCR and exceptionally created ultrasound strategies were utilized broadly in the human and partly in farm animals, however their utilizing in little ruminant and in goat basically are till now uncommon. We trust that this investigation could contribute in fostering these molecular strategies and empowering different specialists in Theriogenology to direct an ever increasing number of studies in this line.

2. Materials and methods

2.1. Ultrasonography

Forty-five Iraqi singleton pregnant goats from 2-5 years of age had been confirmed by days 50-70 of conception. The sex of the fetuses were determined by the location of the genital tubercles[8], when female fetuses had the tubercles closer the tail and male fetuses had the tubercles closer the umbilical cords[9]. The sexing of all identified fetuses was done in conjunction with scanning to ascertain the numbers of fetuses. During the inspection, the location of a genital tubercle was precisely determined. The pregnancy was diagnosed using a real-time B-mode ultrasonic scanner with a 5 MHz external probe[10] (Figure 1). Prior to actually scanning early the next morning, water and food were denied for 12 hours. Scanning was done in the animal's fleece-less inguinal area. One person slightly held the animal against the fence in a standing stance. Just a moment of scan for correct instrument placement, one of goat's hindquarters was packed away. Every session, an ultrasonic partnering gel was placed on the probe for ensure proper friction and remove air from between probes and the animal's skin.

2.2. Primers

The male type (SRY gene) PCR primers were designed in this study utilizing NCBI-Pick primers (AY604733.1), whereas the female type (AMLX gene) PCR primers were designed according to[11], and these primers were provided by (Scientific Resercher Co.Ltd/ Iraq), as shown in table 1

| Primers       | Sequence (5'-3')       | Amplicon |
|---------------|------------------------|----------|
| Female type   | F AATGGTTCGAGCGAAAATGGC| 104bp    |
| AML-X gene    | R TGGACTAACCGAATCACTGAGC|          |
| Male type Sry gene | F ATGAAATAGAACGGTGCAATCG| 160bp    |
|               | R GAAGAGGTTTCCCCAAGGC  |          |
2.3. Blood specimens' collection

Whole blood specimens were collected from the jugular veins of the same 45 pregnant goats examined by ultrasonography. Samples were collected just post ultrasound diagnosis placed in a tube containing EDTA and left outside the cooling for 10 to 20 minutes, then placed in a cooled incubator and then transported to the laboratory for freezing, until the collection of all 45 specimens were completed and utilizing DNA extraction and consequent real time PCR.

2.3.1. Genomic DNA extraction. Genomic DNA from paraffin inserted block tissue tests were separated by utilizing G-spin TM Absolute DNA Extraction Pack (Blood convention) and done by organization directions

2.3.2. Genomic DNA estimation. The removed genomic DNA was by utilizing Nano drop spectrophotometer (THERMO. USA), that check and estimation the immaculateness of DNA through perusing the absorbance in at (260/280 nm).

2.3.3. Real-Time PCR. Real time PCR procedure was performed for high delicate assurance of Female kind (AML-X quality) and Male sort (Sry quality) in pregnant does' blood tests. This strategy was done by technique depicted by as following advances.

2.4. Real-Time PCR Data analysis

qPCR information examination was performed by investigation of limit cycle number (CT esteem) that introduced the positive enhancement Progressively PCR cycle number.

2.5. Statistical analysis

All of the data in this study was recorded as a number and a percentage. To compare the effectiveness of pregnancy detection procedures, a Chi-square test (X²) was used (ultrasonography and PCR technique). The statistical significance level was set to alpha = 0.05 (α = 0.05). A statistically
significant value of $P < 0.05$ was used. The SPSS computer software version 27 was used to compile all of the statistics[12].

3. Results and discussion

Utilizing ultrasonography for early finding of fetal goat sex had been showed a level of accomplishment conclusion of 51.11% (23/45 pregnant does) versus bogus determination in a level of 48.89% (22/45) partitioned into (17) male and (5) female (Table 2). The ultrasonography fetal sexing had been relied upon the restriction of the genital tubercle[8]. These outcomes are higher than that of [13] who uncovered a genuine finding at a level of 30% in a 55 days incubation period, yet the rate was lesser than the aftereffects of similar specialists in a growth time of 65 days when they recorded 90%, while we acquired a level of 71.42% in a similar development period. Our outcomes had been affirmed by the unmistakable sex of the off springs after birth. The gestation time of does in this study were gone from 50-70 days of pregnancy, depending on the past finding of[14] who proposed that fetal sexing in caprine species is conceivable beginning from the 55th day of growth in embryos created by ordinary mating.

Table 2. Results of both diagnostic techniques.

| Techniques  | Total numbers | True diagnosis | False diagnosis |
|------------|---------------|----------------|-----------------|
|            | Sum           | %              | sum            | %              |
| ultrasonography | 45            | 23             | 51.11          | 22             | 48.89          |
| PCR        | 45            | 44             | 97.78          | 1              | 2.22           |
| X2         |               |                | 25.75          |                | < 0.0001*      |
| P value    |               |                |                |                |                |

*Highly significant difference ($P < 0.01$)

The absolute of the fetal sex were 51.11%, which overall lesser than that of[15], who revealed a rate (94%) and (100%) in singleton pregnant does separately, and furthermore lesser than the consequences of[16], who recorded an exactness of 75% and 100% in does at development time of 55 and 65 individually, and they proposed that the bogus determination particularly in the male kidding might be because of the deferred relocation of genital tubercle happened later in the male fetus, which were at first analyzed as females, their idea may clarify the more noteworthy level of bogus analysis in our investigation in regard to the high male fetus number in our inspection in regard to female fetuses (Tables 2 and 3), particularly in the event that we realize that the male kidding typically finished their genital tubercles movement later than female once potentially in light of the more prominent distance to be crossed by the genital tubercle in male fetuses[17]. Then again, The misdiagnosis of female fetuses as male could be because of designs with comparable echogenicity that were confused with the genital tubercle, similar to the edge of the wrinkled rear appendages or maybe even the umbilical cord wrapped around the midsection, as well as the misdiagnosis could happen regularly if investigation have been made too soon[18], nevertheless the trouble of controlling the uterus of sheep and goat during a ultrasonographic examination which influenced the precision of this method[19].
Table 3. Total results of the male fetuses.

| Techniques     | Total male fetuses | True diagnosis | False diagnosis |
|----------------|-------------------|----------------|-----------------|
|                | sum   | %    | sum   | %    |
| ultrasonography| 33    | 16   | 51.52 |
| PCR            | 33    | 32   | 96.97 |
|                |       |      | 1    | 3.03 |

X2 = 19.55
P value = < 0.0001*

* Highly significant difference (P < 0.01)

Table 4. Total results of the female fetuses.

| Techniques     | Total female fetuses | True diagnosis | False diagnosis |
|----------------|----------------------|----------------|-----------------|
|                | sum   | %    | sum   | %    |
| ultrasonography| 12    | 7    | 58.33 | 5    |
| PCR            | 12    | 12   | 100   | 0    |
|                |       |      | 6.31  | 0    |

X2 = 0.012*

*Significant difference (P < 0.05)

DNA has been found in maternal plasma and serum in human, and endeavors have been made to analyze fetal genders utilizing maternal serum and plasma in a request to make a novel noninvasive methods for pre-birth analysis[7]. Fetal DNA is thought to make up somewhere in the range of 3% and 13% of the all-out coursing free DNA in the plasma of pregnant ladies[18]. Albeit the instrument of fetal DNA spillage to maternal dissemination is obscure, cell lysis brought about by physical and immunologic harm, just as formatively directed fetal tissue passing, could permit fetal DNA to infiltrate the placental layer[20]. Chang et al. (2006)[21] exhibited that Amelogenin intensification by PCR is a solid methodology for deciding sex in caprine in an earlier report. In our study we attempt to early analyze the fetal sex in Iraqi pregnant does by utilizing a duplex real time PCR to distinguish the SRY and AMLX qualities in a solitary groundwork in the maternal free fetal DNA at a similar incubation period above we tried by utilizing ultrasonography. We acquired a high precision in fetal sex analysis came to 97.78% (44/45) singleton pregnant does (Table 1) isolated as 96.96% (32/33) male hatchlings and 100% (12/12) female embryos (Tables 2,3). These outcomes are almost like consequences of[22], who recorded a level of precision (94.7%) when they utilizing a traditional PCR to identify AMLX and AMLY in caprine. When contrasting our outcomes concurring with the sex of the analyzed births, they were higher than that of [23] on similar kid species, when they record an exactness of 60 and 40% for female and male individually versus 100 and 96.96 % for female and male embryos separately in our outcomes. These distinctions in the precision of analysis may be due to the low number of tests they utilized in contrast with our examples, and the fluctuation in the technique of DNA extraction utilized in the two examinations. At the point when we study the exactness of the fetal sex analysis as indicated by the time of development, we tracked down that the rates were 100% in the period from day 50 until 65 of incubation, however the rate had been diminished to 95.23% in the period from 66-70 days of growth. This outcome isn't better than finding of [22], who propose that the exactness of fetal sexing increments with advancing pregnancy because of rise of fetal DNA in the maternal flow.
Table 5. Diagnosis according to different periods of gestation.

| Technique       | Days pregnancy 58-62 | Days pregnancy 63-67 | Days pregnancy 68-73 | X2    | P. value |
|-----------------|----------------------|----------------------|----------------------|-------|----------|
|                 | No. =13              | No. =27              | No. =5               |       |          |
| ultrasonography |                       |                      |                      |       |          |
| No.             | %                    | No.                 | %                    |       |          |
| 8               | 61.54                | 11                  | 40.74                | 3.398 | 0.682*   |
| PCR             | 13                   | 26                  | 96.3                 | 5     | 0.682    |
| X2              | 6.19                 | 19.31               | 1.11                 |       | 0.711*   |
| P.value         | 0.013**              | 0.304               | 0.292*               |       |          |

* No significant difference (P > 0.05)
** Significant difference (P < 0.05)
*** Highly significant difference (P < 0.01)

This advanced method had been applied in human[7] and a few individual animal varieties like sheep [3], camel [4], goat[5], ovine and bovine [6].

It is obvious from the above aftereffects of this examination, that the unconventional technique for fetal sex analysis is profoundly productive and essentially unmistakable than the ultrasonography in various times of incubation tried, sexual orientation of embryos, and altogether fetal sexing by utilizing progressive strategy (Tables 1,2,3,4,). Another benefit of this strategy in fetal sex assurance is that quicker, requiring no skill in contrast with the ultrasonography.

4. Conclusions
Successful utilization of real time PCR for early determination of fetal sex in singleton pregnant does with extremely high level of accuracy. Utilizing ultrasonography with gained productivity in early fetal sexing in singleton pregnant does. The real time PCR strategies for fetal sexing in singleton pregnant does is more exact and productive and quicker than the ultrasonography. The best percentage of accuracy of ultrasonography in singleton pregnant does in our study was in 68-73 days of gestation.

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