

# Two Corpora Lutea Seen at 6–13 Weeks' Gestation Infers Dizygoty Among Spontaneous Same-Sexed Dichorionic Twins

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Current ultrasound techniques can accurately determine the chorionicity of twins, but not zygosity. We previously proposed that the zygosity of spontaneously conceived twins can be determined at early ultrasound, where 2 corpora lutea infers dizygoty, and 1 implies monozygoty. Here we did a case series, comparing zygosity predicted using this method with definitive DNA genotyping of twins after birth. We retrospectively identified 14 ultrasound reports of spontaneous twin pregnancies at 6(+0 days) to 13+6 weeks' gestation, where both ovaries were seen and the number of corpora lutea documented. We visited all twin pairs, obtained buccal smears, and determined zygosity by genotyping 9 independent microsatellite markers. All 8 cases where 2 corpora lutea were seen were dizygotic pregnancies. One further case where 3 corpora lutea were seen was also dizygotic. All 3 sets of monozygotic twins had 1 corpus luteum. There were 2 cases incorrectly assigned, where 1 corpus luteum was seen in dizygotic pregnancies. We conclude if 2 corpora lutea are seen at a first trimester ultrasound of spontaneously conceived dichorionic twins, they appear to be almost certainly dizygotic. However, if 1 corpus luteum is seen in dichorionic twins, zygosity cannot be determined with certainty since it is either monozygotic, or dizygotic where a second corpus luteum has been missed.

**Keywords:** Zygosity, ultrasound, chorionicity, twins, corpus luteum

Twins and those around them are keenly interested whether or not they are identical. This knowledge is used lifelong to shape identities and form social networks (Kyvik & Derom, 2006). Since it is chorionicity, not zygosity that appears to confer a differential level of obstetric risk, the issue of zygosity is either briefly, or often never addressed during antenatal care.

Worse still, there seems to be a common misconception among the wider population that the presence of 'two sacs' on ultrasound confers the diagnosis of fraternal twinning (Yahoo! Inc., 2007). While monochorionic twins are almost always identical, same-sexed dichorionic twins can be either dizygotic, or monozygotic. Using a combination of fetal sex and chorionicity, zygosity can be determined by ultrasound using traditional indicators (monochorionic placentation, opposite sex) in only 55% to 65% of spontaneous twin pregnancies (Derom et al., 2001).

We have previously proposed (Tong et al., 2004) that zygosity of spontaneous twins can be determined at a first trimester ultrasound by counting the number of corpora lutea. Since the corpus luteum forms at the site of egg release, one corpus luteum would suggest monozygosity whereas two would imply dizygoty. However, this method of zygosity determination has never been validated comparing ultrasound findings with the gold standard of DNA genotyping.

Here we report a retrospective case series, correlating zygosity of spontaneous twins predicted by ultrasound using this method with definitive zygosity determination by DNA analysis.

## Materials and Methods

### Study Participants

We did a retrospective case series where we identified ultrasound reports of viable twins at 6(+0 days) to 13+6 weeks' gestation. The inclusion criteria were women with spontaneous twin pregnancies where

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both ovaries were seen. At our center, we routinely document the presence of corpora lutea in early pregnancy scans and make a comment on the report if the referring doctor stated the twins were iatrogenic. We contacted the referring obstetrician to ensure the women had given birth to two live babies. After this, we contacted these women, confirmed that their twins were in fact spontaneous, then invited them to participate. We obtained ethics approval before commencing this study (Epworth Hospital Human Research Ethics Committee).

#### Ultrasound Assessment of the Presence of Corpora Lutea

All examinations were transvaginal, and performed by experienced obstetric ultrasonographers. Advanced technology Laboratories™ HDI 5000 ultrasound machines were used for all ultrasound scans. After confirmation of a live intrauterine pregnancy, measurements were taken to confirm gestational age. The ovaries were then identified by transvaginal B-mode ultrasonography. Once localized, color and power Doppler sonography using the low velocity setting (10cm/sec) was applied to identify the peripheral ring of vascularity which characterizes the CL. CL diameter was calculated as the maximum transverse diameter obtained after examination in two planes.

#### Assignment of Zygoty

Twins with opposite gender were assigned as dizygotic. We used DNA analysis to determine zygoty in all remaining cases, irrespective of chorionicity. A buccal smear was taken from each twin, the genomic DNA isolated and zygoty determined by typing nine independent microsallele markers plus the sex marker amelogenin (AmpFLSTRw Profiler Plus™, Applied Biosystems, Foster City, CA, USA). The probability of dizygosity given concordance of all markers in the panel was  $< 10^{-4}$ . Those who did the DNA analysis were blinded as to the ultrasound results.

#### Results

Fourteen pairs of spontaneous twins were recruited where the ovaries were identified on ultrasound. There were eleven pairs of dizygotic twins and three pairs of monozygotic twins (see Table 1). All eleven dichorionic twins in our cohort were dizygotic. There were nine cases where two or more corpora lutea were observed, meaning zygoty would have been determined correctly for these cases using the method we propose. Four were same-sexed twin pairs, where current ultrasound parameters would not have been able to assign zygoty. A single corpus luteum was reported for the remaining two cases of dichorionic twins.

There were three cases of monochorionic twins. One corpus luteum was seen in all these cases, in agreement with the diagnosis of monozygoty.

#### Discussion

Our results suggests if two or more corpora lutea are seen in same sexed spontaneous dichorionic twins

**Table 1**

Zygoty, Chorionicity and Number of Corpora Lutea Among 14 Twin Pairs

Gestation at ultrasound Weeks (+days)	Chorionicity	Gender	Corpora lutea (number)
<b>Monochorionic</b>			
9(+5)	Monochorionic	2 males	1
12(+2)	Monochorionic	2 females	1
12(+3)	Monochorionic	2 males	1
<b>Dichorionic</b>			
6(+0)	Dichorionic	Male/female	2
8(+0)	Dichorionic	Male/female	1*
12(+0)	Dichorionic	Male/female	3
12(+0)	Dichorionic	2 females	2
12(+1)	Dichorionic	Male/female	2
12(+2)	Dichorionic	Male/female	2
12(+2)	Dichorionic	Male/female	2
12(+2)	Dichorionic	2 females	2
13(+2)	Dichorionic	Male/female	2
12(+5)	Dichorionic	2 females	1*
12(+6)	Dichorionic	2 females	2

Note \*Incorrect assignment of zygoty

(where both ovaries are clearly visualized), the twins will be dizygotic. While we report limited numbers in our case series and did not include cases of dichorionic monozygotic twins, both the biological plausibility and fact that this approach is 100% accurate lends strong credence that two corpora lutea implies almost certainly dizygosity. However, zygoty cannot be assigned if one corpus luteum is seen since it could be either monozygotic, or dizygotic where perhaps a second corpus luteum was missed.

While the the corpus luteum can be reliably seen in almost all cases of spontaneous singleton pregnancies at 5(+0 days) to 9+6 weeks gestation (Tong et al., 2002), it can only be detected in 82% of cases at 10+0 to 13+6 weeks' gestation (Rowan et al., 2007). However, this observation is not at odds with, but agrees with our conclusion that zygoty cannot be assigned from the presence of one corpus luteum, but the presence of two strongly implies dizygosity.

Since the corpus luteum is reliably seen before 10 weeks' gestation (Tong et al., 2002) but may be missed after this gestational age (Rowan et al., 2007), it is possible that between 5+0 to 9+6 weeks' gestation, the presence of one corpus luteum reliably indicates monozygoty and excludes the possibility of dizygosity. However, this would need to be proven in a formal prospective study with researchers well practiced at characterizing the corpus luteum. Such a study would be difficult to undertake, principally because spontaneous twinning is a relatively rare event, occurring at around 12 per 1000 pregnancies (Bulmer, 1970). It would be very difficult to maintain awareness among

staff that such a prospective study is recruiting in the face of small numbers of eligible participants presenting for ultrasound scans.

In conclusion, we have found that same sexed dichorionic twin pairs with two corpora lutea noted on ultrasound are likely to be dizygotic twins.

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