Reproductive characteristics of female white-tailed deer (Odocoileus virginianus) in the Midwestern USA

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Abstract
Knowledge of reproductive characteristics of wild populations is necessary to inform responsible management decisions that promote herd health. As management, goals, and free-ranging populations change over time and landscapes, updated knowledge of reproductive characteristics are needed to inform responsible management practices. We estimated reproductive characteristics of female white-tailed deer in Illinois, including pregnancy rate, litter size, fetal growth and fetal sex ratio. We found maternal age to have an important influence on several reproductive factors. Approximately 66% of tested females (n = 3884) were pregnant and pregnancy rates increased with increasing maternal age, from 20.5% in fawns to 85.8% in adult deer. Litter size ranged from 1 to 5 fetuses per pregnant female. The average litter size was 1.9 ± 0.54 fetuses per pregnant female and also increased with age, from 1.2 in fawns to 2.0 in adults, respectively. Breeding season peaked in November with the mean estimated conception dates of fetuses varying with maternal age. Fawns conceived fetuses later in the breeding season (December 2) compared to yearlings and adults (November 11 and 8, respectively). We measured the body mass index (BMI) of all fetuses and found that litter size and female age influence fetal size. We found no bias in fetal sex ratio (average 1.0:1.0, male:female) but we observed a sex bias in fetal size (mean BMI male = 0.71, female 0.67) across all maternal age classes. A comparison of the current study and previous reports indicate that variation in maternal age within a population is an important driver of reproductive metrics, likely because maternal age and body size or condition are related. Furthermore, variation in resource availability will influence reproductive rates, especially among fawn females.

1. Introduction
North American cervids, including white-tailed deer (Odocoileus virginianus), mule deer (O. hemionus), elk (Cervus canadensis), and moose (Alces alces) are recreationally valuable and popular game species. The Odocoileus genus has the highest numbers, highest economic value [1], and is found across a majority of North America [2]. While white-tailed deer are among the most abundant species presently, unregulated hunting threatened to eliminate this species from much of its range in the early 1900’s [3]. Since that time, white-tailed deer have been managed extensively and recent decades have seen dynamic changes in white-tailed deer populations. For example, deer populations in the United States and Canada in 1948 were estimated at only about 6 million deer [4], whereas in 2015 that same number was actually harvested by hunters during legal hunting seasons [5].

Population management of wildlife species, especially large game species requires knowledge of population dynamics relative to carrying capacity of the landscape. Further, studies of other cervids have indicated that population density can influence female reproduction [6]. Reproductive parameters such as pregnancy rates, birth rates, neonate and juvenile survival (i.e., fawn recruitment), and litter size are important variables that can be used to model
population growth or decline and ultimately guide management decisions. Given the dynamic changes in deer populations in the recent past, and the importance of having contemporary estimates of reproductive parameters to monitor variations in deer populations and guide management decisions, the purpose of this paper is to examine the reproductive characteristics of female white-tailed deer using a large dataset beginning in 2003.

The upper Midwest landscape is a mixture of fertile agricultural cropland interspersed with forests. Illinois has a long history of deer management and previous studies have investigated white-tailed deer reproduction [7,8]. However, few data on reproduction have been reported in recent years. Previous estimates of reproductive parameters may not be applicable if deer population size, habitat quality and quantity, or food resources have changed substantially over time [9]. Therefore, additional research is warranted to provide updated estimates of reproductive parameters. In order to update this information, we examined baseline reproductive characteristics of female white-tailed deer based on a large sample set obtained over a decade of sampling. Specifically, we investigated (1) pregnancy rate and estimated conception peaks, (2) litter size and sex ratio, and (3) fetal growth.

1.1. Species characteristics

White-tailed deer are seasonal breeders. Estrus and mating occurs during the rutting season, which in central Illinois ranges from October to December. With an average gestational length of 200 days [10], parturition follows from May to July [7]. Although at a later date than older females, fawns may enter estrus in the autumn of their first year [10]. Female white-tailed deer gain more than half of their adult mass of 60 kg between 0.5 yr and 2–4 yrs [11], when they attain maximum body size [10]. Males do not reach stable body mass of 80 kg until approximately 5 years [10].

1.2. Pregnancy rate and estimated conception date

Research of white-tailed deer and roe deer (Capreolus capreolus) indicates pregnancy rates increase with maternal age [8,12,13]. Maternal age and reproduction are linked as a high plane of nutrition (defined as the quantity and quality of per capita food intake), results in rapid growth and earlier maturity (i.e., estrus) in many species [14,15]. Conversely, females in less-than-average body condition may exhibit lower pregnancy rates. In the wild, fawn breeding rates vary with habitat quality [16] and deer habitat in Illinois generally is considered high quality [9] leading to an expectation of relatively high fawn pregnancy rates although yearly variation is expected given variation in weather conditions for plant growth (resource availability). Older females tend to conceive earlier than younger females [12] and we therefore expect the estimated breeding dates of older females to occur earlier than fawns and yearlings.

1.3. Litter size and sex ratio

White-tailed deer typically carry one or two fetuses although litter size varies with maternal age [10]. Fawns in southern Illinois (based on fetal counts) produced a single offspring compared to yearlings and adults that had higher proportions of twin and triplet litters [8]. Litter size, similar to pregnancy rate, is connected to maternal age through body condition. Because of the high metabolic cost of pregnancy, it is reasonable to expect females with larger body size (i.e., more fat) to be better suited to tolerate body fat loss, and thus multiple fetuses per pregnancy, compared to females of smaller body size. Therefore, we expect litter size to increase with maternal age among Illinois deer.

Fetal sex ratios of white-tailed deer appear responsive to maternal age and environmental conditions but the literature is divided on the interpretation of available studies [17–20]. A previous study of white-tailed deer at least 1 yr old, indicated that the percentage of male fawns declined with an increase in maternal age and litter size [17]. In Ohio, fawn and yearling females carried more male fetuses, but the sex ratio was balanced among adult females [21]. A study in Missouri found older females have more male offspring [22] whereas fetal sex ratios favored females among adult females in an Ohio farmland region [23]. Because our study area includes agricultural habitat with abundant food resources, we expect females in our study area to be in relatively good body condition and to carry a lower proportion of male offspring compared to yearlings. We expect the proportion of males to decrease with increasing litter size within an age class.

1.4. Fetal growth

White-tailed deer fetal growth is highly predictable from approximately 40 days post-conception until parturition [24]. Descriptive growth keys (e.g., Carnegie stages) for white-tailed deer provide accurate age estimates based on morphological development [25,26]. Although descriptive keys may be slightly more exact, crown-rump length measurements afford a method that requires less detailed observation to provide an accurate age and conception date estimate [25]. Several factors influence birth size across most mammals including age, size and body reserves of the female at conception, and litter size [27]. Maternal size influences fetal growth because traits that influence growth are heritable and maternal size is determined by female body condition (i.e., nutrition and percent body fat), which in turn governs fetal size. Among white-tailed deer, Cothren et al. [28] indicated that maternal age, maternal weight or the number of fetuses can impact fetal growth rates after accounting for fetal age. Therefore, we expect fetal size to increase with maternal age and decrease with increasing litter size and male fetuses are expected to be larger than females [29,30].

2. Materials and methods

Live animal work was conducted under the oversight of the Illinois Department of Natural Resources. Laboratory work was conducted under the oversight of the University of Illinois Institutional Biosafety Committee.

2.1. Sampling

White-tailed deer embryos and fetuses were obtained through the state of Illinois chronic wasting disease (CWD) control program, an Illinois Department of Natural Resources (IDNR) program. Samples were collected by IDNR personnel from 15 northern Illinois counties each year from 2003 through 2013 during January through March (Fig. 1). The county where each deer was killed was recorded and used as the geographic location for all analyses. Age of each female deer was estimated based on sequential development of dentition [31]. Deer were classified into age groups using management terminology as fawn (8–11 months at time of sampling), yearling (20–23 months at time of sampling) or adult (>32 months at time of sampling). Deer were brought to one of several IDNR laboratories and processed to allow donation to local food banks after disease testing. During processing, IDNR biologists measured crown-rump length, crown-rump width, crown-rump height and crown-rump depth for each fetus.
length (cm), determined sex by direct observation of genitalia, and collected tissue for other studies.

2.2. Pregnancy rate and estimated conception date

The ability to detect a fetus depends on fetal development and size. Because of difficulty in detecting fetuses in early pregnancy, we fit a sigmoid curve to all data and determined when our ability to detect fetuses was constant (Fig. 2). Subsequent analyses were conducted using samples collected after February 1 (Fig. 2, vertical reference line) to avoid bias resulting from non-detection.

All statistical analyses were completed using SAS v. 9.4 (Cary, N.C.). We used a factorial logistic regression (PROC LOGISTIC) to determine whether pregnancy was influenced by maternal age (fawn, yearling, adult; treated as factor throughout), year (treated as factor throughout), geographic location (treated as factor throughout), or any two-way interactions. We tested maternal age because body condition likely changes as females grow. We also included year and geographic location as covariates because temporal and spatial variation in resource availability have the potential to contribute to the body condition of females. We did not include day of sampling (number of days into the year) in predictive models for pregnancy, litter size or fetal sex ratio because there was no correlation with those variables (Pearson’s correlation coefficient: pregnancy \( r_{3383} = 0.02, P = 0.16 \); litter size \( r_{3887} = 0.01, P = 0.62 \); fetal sex ratio \( r_{2542} = 0.02, P = 0.45 \)).

Because it is impossible to observe large fractions of a population in the wild, wildlife studies rely on fetal developmental milestones or regression analysis to estimate dates of conception. There are potential errors in each of these approaches and we utilized a widely used regression calculated from captive breeding data [25]. We calculated fetal age in days (FAD) based on a predictive equation for white-tailed deer (FAD = 36.82 + (0.32 crown-rump length)) which is expected to be accurate within \(-2.4\) and \(+4.9\) days [25]. We then estimated the conception date for each fetus by subtracting fetal age from the collection date and used the mean estimated conception date for all fetuses within a litter when appropriate. We evaluated whether maternal age influenced estimated mean conception date using an ANOVA with means separation (using PDIF in PROC GLM). We included year, geographic location, litter size and all two-way interactions as covariates to capture variation that may contribute to female body condition.

2.3. Litter size and sex ratio

All fetuses were included in litter size calculations but fetuses with undetermined sex \( (n = 58) \) were omitted from analyses as appropriate. To determine whether litter size varied with age class, we used a Poisson regression with a log link function (PROC GENMOD). The dependent variable was litter size and the independent variables tested were maternal age, year collected, geographic location of collection and all two-way interactions. Each pregnant female was considered an observation. We included maternal age because it likely represents some variation in maternal body size/condition. We included year and geographic location because food and nutrient availability leading to maternal condition may vary with each. All non-pregnant females were removed from analyses of litter size. Large litter sizes were relatively rare events in each age class (fawn triplets \( [n = 1]\), yearling quadruplets \( [n = 3]\), and adult quintuplets \( [n = 1; \text{Fig. 2b}]\)). To focus on typical reproductive events and avoid skewing statistical evaluation of normal litter sizes, we removed the rare events from analyses.
The sex ratio of fetuses (male fetuses/total fetuses) was determined for all pregnant females. To determine whether fetal sex ratio varied with female age on a population level, we considered all fetuses as unique observations. We used a binary logistic regression model to test the probability of a fetus being male regardless of litter size (PROC LOGISTIC). Because maternal body condition may influence fetal sex ratio, we included all variables that could influence maternal body condition: maternal age, year, geographic location, and litter size. We also included day of sampling to account for variation in gestational age and all two-way interactions as predictors of fetal sex ratio. Furthermore, we conducted a second analysis in which we included only observations from compound litters (>1 fetus) in order to assess within-litter fetal sex ratios. We used a cumulative logistic regression model to test whether the mean crown-rump length of the litter (used to estimate conception date), maternal age, year, geographic location, and litter size. We also included day of sampling, maternal age, litter size, year, geographic location, and the interaction of maternal age with litter size. Day of sampling is the day during gestation when each female was collected, rather than an estimate of length of gestation (fetal age) when collected. Although day of sampling had some correlation with crown-rump length, it had a significant independent contribution to the model $R^2$ value and was therefore included in the model. We included maternal age as a predictor because it corresponds to body size and captures the collinear predictors of weight and length that describe allometric growth patterns. The independent variables were day of sampling, maternal age, litter size, year, geographic location, and the interaction of maternal age with litter size. Day of sampling is the day during gestation when each female was collected, rather than an estimate of length of gestation (fetal age) when collected.

2.4. Fetal growth

We identified extreme growth outliers by comparing within-litter growth variation (crown-rump length) to the observed growth variation of all fetuses. To do this we determined the overall interquartile (IQ) range of crown-rump lengths from compound litters (>1 fetus) and excluded litters where the within-litter range exceeded the observed range of the population or exceeded previously reported within-litter variation of 5.1 cm $[32,33]$, corresponding to an estimated difference of 16 days in gestational age based on the predictive equation of Hamilton et al. $[25]$. Identified outliers included compound litters with sibling lengths that appeared biologically implausible relative to each other which possibly resulted from recording or transcription errors over the decade of sample collection. In total we removed 21 litters, <1% of full dataset.

Day of sampling was correlated with fetal body size metrics and retained as a variable in body size models (body mass index $r_{785} = 0.72$, $P < 0.0001$; weight $r_{790} = 0.82$, $P < 0.0001$; crown-rump length $r_{795} = 0.78$, $P < 0.0001$). To determine factors influencing fetal growth, we used body mass index (BMI) of individual fetuses as a metric of growth assessed using an ordinary linear regression model (PROC GLM). BMI (weight/crown-rump length$^2$) is a popular indicator of body condition because it is relatively easy to gather robust data, and it can serve as a good measurement of population-level body size $[34]$. We selected BMI because it captures the collinear predictors of weight and length that describe allometric growth patterns. The independent variables were day of sampling, maternal age, litter size, year, geographic location, and the interaction of maternal age with litter size. Day of sampling is the day during gestation when each female was collected, rather than an estimate of length of gestation (fetal age) when collected. Although day of sampling had some correlation with crown-rump length, it had a significant independent contribution to the model $R^2$ value and was therefore included in the model. We included maternal age as a predictor because it corresponds to body size among females, and we included litter size because the number of fetuses reportedly can influence sex ratio and therefore, fetal size $[35]$. Year and geographic location were included to account for variation in food and nutrient availability that may influence maternal body condition.

We examined BMI values to determine whether male and female fetuses were the same size within a litter at a given stage of development. In order to test whether fetal size (BMI) was influenced by sex we nested fetal sex within mother in order to compare multiple fetuses from the same litter. We tested this by maternal age class to determine whether maternal age influenced differences between fetal sizes of males and females. We also independently tested whether fetal sex (nested within mother) had a greater influence on fetal weight or length. For all glm tests, Bonferroni corrections of multiple comparisons were applied for comparisons of significant differences.

3. Results

3.1. Pregnancy rate and estimated conception date

Our dataset for analyses included 3884 females and 4781 fetuses (Table 1). On average, 65% of females were pregnant. Maternal age ($P < 0.0001$), year ($P < 0.0001$) and the interaction of maternal age and year ($P < 0.0001$) influenced pregnancy rates (overall model Nagelkerke’s $R^2$ = 0.49, likelihood ratio $X^2_{32} = 1695.07, P < 0.0001$). More yearlings were pregnant than fawns and more adults were pregnant than yearlings ($Table 1; X^2_{10} = 132.4, P < 0.0001$) and geographic location ($X^2_{11} = 61.1, P < 0.0001$).

The mean conception date of all fetuses was estimated to be November 10 (314.7 days into the year; SE 0.28). Estimated conception date was influenced by maternal age which accounted for the majority of the variation ($P < 0.001, \eta^2 = 0.082$) followed by year ($P < 0.001, \eta^2 = 0.034$) and litter size ($P < 0.001, \eta^2 = 0.029$). The mean estimated conception date of adult and yearling fetuses was November 8 (311.9 days; SE 0.24).
and November 11 (315.4 days; SE 0.62), respectively. Fawn females conceived later, at a mean of December 2 (336.3 days; SE 1.37).

### 3.2. Litter size and sex ratio

The mean litter size among all pregnant females was 1.9 fetuses (Table 1). Rare events of large litters (n = 5) were removed from analysis, but summary statistics are presented in Table 2. Overall, yearling and adult female deer most commonly had twin litters while fawns had single offspring (Fig. 2b). Maternal age class was the only significant predictor of litter size ($\chi^2 = 75.35, P < 0.0001$). Fawns had the smallest litter sizes (1.2 fetuses/female) followed by yearlings (1.8 fetuses/female) and adults had the largest (2.0 fetuses/female).

On a population level, we did not find any significant predictors of fetal sex ratio. Neither did we find any significant predictors of fetal sex ratio when testing within-litter fetal sex ratios of compound litters only. Maternal age was not a significant predictor of fetal sex ratio. Over time (Table 1) there was no difference between age classes with an average fetal sex ratio of 1:1.0 (male:female). For different years, the sex ratios of fawn mothers ranged from 33 to 68% males, yearling mothers ranged from 38 to 87% males, and adult mothers from 45 to 54% males (Table 1). While we did observe a numerical decline in the proportion of males with increasing litter size, which is correlated with maternal age, the trend was not significant (Table 3). Neither maternal age ($P = 0.08$) nor litter size ($P = 0.97$) were significant explanatory variables.

### Table 1

Total number of female deer sampled and reproductive characteristics of female white-tailed deer in northern Illinois, 2003–2013.

<table>
<thead>
<tr>
<th>Fiscal year</th>
<th>Maternal age class</th>
<th>Total ($n$)</th>
<th>Pregnant ($n$)</th>
<th>Fetuses ($n$)</th>
<th>Pregnancy rate (%)</th>
<th>Fetal sex ratio 2</th>
<th>Mean litter size of pregnant females</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>Fawn</td>
<td>36</td>
<td>3</td>
<td>3</td>
<td>8.3</td>
<td>0.33 [0.41, 0.53]</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Yearling</td>
<td>17</td>
<td>4</td>
<td>6</td>
<td>23.5</td>
<td>0.38 [0.34, 0.42]</td>
<td>1.5</td>
</tr>
<tr>
<td>2004</td>
<td>Fawn</td>
<td>77</td>
<td>29</td>
<td>58</td>
<td>37.7</td>
<td>0.53 [0.48, 0.57]</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>Adult</td>
<td>100</td>
<td>26</td>
<td>30</td>
<td>26.0</td>
<td>0.43 [0.40, 0.46]</td>
<td>1.2</td>
</tr>
<tr>
<td>2005</td>
<td>Fawn</td>
<td>55</td>
<td>30</td>
<td>57</td>
<td>54.6</td>
<td>0.87 [0.83, 0.90]</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>Yearling</td>
<td>191</td>
<td>152</td>
<td>298</td>
<td>79.6</td>
<td>0.48 [0.46, 0.50]</td>
<td>2.0</td>
</tr>
<tr>
<td>2006</td>
<td>Fawn</td>
<td>104</td>
<td>20</td>
<td>23</td>
<td>19.2</td>
<td>0.50 [0.47, 0.53]</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>Yearling</td>
<td>35</td>
<td>29</td>
<td>51</td>
<td>82.9</td>
<td>0.64 [0.61, 0.68]</td>
<td>1.8</td>
</tr>
<tr>
<td>2007</td>
<td>Fawn</td>
<td>119</td>
<td>24</td>
<td>31</td>
<td>20.2</td>
<td>0.46 [0.44, 0.48]</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>Yearling</td>
<td>39</td>
<td>29</td>
<td>47</td>
<td>59.2</td>
<td>0.70 [0.68, 0.72]</td>
<td>1.9</td>
</tr>
<tr>
<td>2008</td>
<td>Fawn</td>
<td>1137</td>
<td>233</td>
<td>283</td>
<td>20.5 ± 0.8b</td>
<td>0.50 [0.41, 0.53]</td>
<td>1.2 ± 0.42b</td>
</tr>
<tr>
<td></td>
<td>Yearling</td>
<td>562</td>
<td>436</td>
<td>780</td>
<td>77.6 ± 20.3b</td>
<td>0.52 [0.47, 0.54]</td>
<td>1.8 ± 0.55b</td>
</tr>
<tr>
<td>2009</td>
<td>Fawn</td>
<td>3884</td>
<td>2543</td>
<td>4781</td>
<td>65.5 ± 31.4</td>
<td>0.51 [0.49, 0.52]</td>
<td>1.9 ± 0.54</td>
</tr>
<tr>
<td></td>
<td>Yearling</td>
<td>177</td>
<td>149</td>
<td>294</td>
<td>84.2</td>
<td>0.49 [0.46, 0.51]</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Adult</td>
<td>2185</td>
<td>1874</td>
<td>3718</td>
<td>85.8 ± 16.0b</td>
<td>0.51 [0.49, 0.52]</td>
<td>2.0 ± 0.49b</td>
</tr>
</tbody>
</table>

1Total number of females sampled; includes only deer sampled after 01 February to account for potentially undetected fetuses.
2Number of male fetuses divided by the total number of fetuses. Fetuses with undetermined sex omitted.
3Values within a column with different superscript letters differ significantly at $P < 0.001$.
495% confidence limits of the mean in brackets.

### Table 2

Summary statistics of rare, large litters relative to maternal age. Fetuses were recovered from white-tailed deer in Illinois. Age reflects maternal age, Date reflects the day each litter was collected, CRL indicates crown-rump length, SE indicates standard error, BMI indicates body mass index.

<table>
<thead>
<tr>
<th>Litter Size</th>
<th>Age</th>
<th>Date</th>
<th>CRL (cm) ± SE</th>
<th>Mass (g) ± SE</th>
<th>BMI ± SE</th>
<th>Sex ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>fawn</td>
<td>4-Feb</td>
<td>12.67 ± 0.17</td>
<td>48.77 ± 0.22</td>
<td>0.3 ± 0.01</td>
<td>2 M:1 F</td>
</tr>
<tr>
<td>4</td>
<td>yearling</td>
<td>7-Feb</td>
<td>20.13 ± 0.69</td>
<td>223.25 ± 10.65</td>
<td>0.54 ± 0.04</td>
<td>3 M:1 F</td>
</tr>
<tr>
<td>4</td>
<td>yearling</td>
<td>16-Mar</td>
<td>26.00 ± 0.54</td>
<td>596 ± 16.08</td>
<td>0.52 ± 0.03</td>
<td>3 M:1 F</td>
</tr>
<tr>
<td>4</td>
<td>yearling</td>
<td>27-Feb</td>
<td>23.50 ± 0.25</td>
<td>412.23 ± 13.25</td>
<td>0.75 ± 0.01</td>
<td>1 M:3 F</td>
</tr>
<tr>
<td>5</td>
<td>adult</td>
<td>7-Mar</td>
<td>26.86 ± 0.40</td>
<td>477.95 ± 44.14</td>
<td>0.66 ± 0.05</td>
<td>2 M:3 F</td>
</tr>
</tbody>
</table>
3.3. Fetal growth

Fig. 3a illustrates developmental stages throughout gestation. In nearly all conceptuses (98.4%), embryonic development, during which all major systems and structures develop, was complete by February 1. A small proportion of conceptuses from each maternal age class was considered embryonic in the data set (1.5% adult, 1.2% yearling, 4.7% of fawn conceptuses). At the February 1 detection cutoff date, crown-rump length of fetuses averaged 14.6 cm, which correlated with fetal development (characterized by growth in length and mass) rather than embryonic development. Once fetuses reached 35–40 cm in crown-rump length, maturation of tissues was complete and minor morphological characteristics such as pigmentation, spots, eyelashes and apparent hair follicles were present (Fig. 3b). When we removed outlier compound litters with sibling lengths that appeared biologically implausible relative to each other, we excluded 9 twin and 12 triplet litters (<1% of full dataset).

The mean BMI of all fetuses was 0.61 (Fig. 4). All variables influenced fetal BMI ($F_{390} = 248.89, P < 0.0001, r^2 = 0.621$). Day of sampling accounted for most of the variation ($P < 0.0001, \eta^2 = 0.502$). BMI tended to increase with increasing maternal age ($P < 0.0001, \eta^2 = 0.0195$), litter size ($P < 0.0001, \eta^2 = 0.008$) and was associated with the interaction of these two variables where BMI increased with litter size in fawns but not in adults or yearlings ($P < 0.0001, \eta^2 = 0.005$). Year ($P < 0.0001, \eta^2 = 0.0431$) and geographic location ($P < 0.0001, \eta^2 = 0.009$) also accounted for variation in fetal BMI.

Male fetuses were larger than female fetuses based on BMI ($F_{3524} = 11.04, P < 0.0001, r^2 = 0.9720$); group mean BMI for males was 0.71 (SE ± 0.01), as compared to 0.67 (SE ± 0.01) for females. Fetal crown-rump length and fetal mass were both correlated with fetal sex (crown-rump length $F_{3530} = 52.15, P < 0.0001, r^2 = 0.9939$; mass $F_{3526} = 33.66, P < 0.0001, r^2 = 0.9906$). Among adult mothers, fetal sex nested within mother was a significant predictor of BMI ($F_{3698} = 10.38, P < 0.0001, r^2 = 0.9683$), similar to yearling mothers ($F_{584} = 10.75, P < 0.0001, r^2 = 0.9723$). We did not test for differences in BMI based on litter size of fawn females because of the large proportion of single fetus pregnancies.

4. Discussion

Resource availability is a central concern to wildlife management because it determines carrying capacity (i.e., the number of animals that can be sustainably supported by any particular landscape or area). It is known that resource availability, in particular nutrition, is a major contributor to reproduction in wild animals. Inadequate nutritional condition may contribute to failure to achieve estrus [36]. The smallest, youngest animals, i.e., fawns, are at the greatest risk from resource competition followed by yearlings which are less competitive than adults. If reproduction is dependent on growth and body condition (fat), fewer animals, especially fawns, on a resource-limited landscape may be able to achieve a body condition that is suitable for estrus. Therefore, from a practical management point, resource availability is reflected in reproduction of the different age classes of deer.

The mean estimated conception date of all fetuses was November 10 which is typical of the Midwest. Because deer are seasonal breeders, photoperiod which is related to latitude, triggers estrus [21,37]. Our results were consistent with previous reports from smaller studies in the Midwest indicating adult female breeding peaked between November 3–16 in Ohio [21] and on November 16 in Missouri [22]. Even with a photoperiod trigger of estrus, estimated conception date varied with maternal age which supports previous reports of later breeding and a flatter distribution of breeding dates among fawns.

The peak of breeding corresponds to a peak in deer activity and movement [38] and a corresponding increase in deer-vehicle accidents. In Illinois from 2005 to 2013, the highest peak in deer-vehicle collisions occurred between November 6 and November 19 (unpublished data) which corresponds roughly to the mean estimated conception date of November 10 in our study.

Both geographic location and year accounted for some variation in pregnancy rates which is reasonable given that each year can result in a unique combination of precipitation and temperature...
that likely influence resource availability. Pregnancy rates in our study were on the low end of the previously reported range. Pregnancy rates of adult white-tailed deer vary from 80% to 100% depending on the study [7,8,12,21,23]. One possible reason for this is the overall variation in habitat type and resource availability across our large study area. For example in Ohio, Stoll and Parker [23] found a higher rate of fawn pregnancy in the farmland (68%) compared to the hill country (51%) although farmland area was ~13% forested compared to the hill country with 51% forested habitat. Clearly, deer reproduction is not dependent solely on the amount of forest in a study area. Our study area was 22,790 km² with considerable variation in the amount of landscape dedicated to agriculture, forest, or urban development, each providing variability in resource quantity and quality.

A second possible explanation relates to sample bias in the smaller studies that used hunter harvest as a source of sample. Hunters generally select larger deer when possible whereas our smaller studies that used hunter-harvest as a source of sample. Previous reports indicated that single fetuses were heavier in later based on conception date estimates from crown-rump length. This was primarily because singleton litters were conceived earlier than twins or triplets [35]. In our study, older females were pregnant with larger fetuses which supports the previous pattern of young females producing smaller fawns compared to older females [8,35].

Fawns may breed as young as 6 months. By reaching first estrus at such a young age, females are able to participate in the first annual breeding season. However, with estrus and mating occurring at such a young age, fawns will be particularly susceptible to reduced reproduction as a result of small body size. Previous work suggested that there is a critical fat/lean biomass ratio that regulates puberty onset in fawns [37]. By fall, fawns may have only gained 50% of their adult weight [44] and their fat stores are lower (Fig. 2) likely contributing to their low embryo-based pregnancy estimates.

Our study supported previous research that white-tailed deer typically carry one or two fetuses. Year and location were important predictors for whether a female was pregnant, but once females were pregnant, there was no effect of year or geographic location on the number of fetuses the female carried. Other studies report average litter sizes in the range of 1.6 [12] to 1.8 [8], similar to our study. In contrast to relatively consistent reports of overall litter size, variation in litter size across maternal age classes varied widely driven by fawn pregnancies. Literature reports of fawn litter sizes are variable in both directions compared to the 21% twins that we observed, ranging from 0% compound litters for fawns in southern Illinois [8] to 29% twins in Ohio [23]. In contrast to fawns, adult litter sizes were similar. Litter size was 1.9 fetuses per adult in southern Illinois [8], 2.0 among adults in Ohio [21] and 1.9 per pregnant adult in Missouri [23].

In our study which included 4781 white tailed deer fetuses collected over ten years, we found a balanced sex ratio. Furthermore, because of the large dataset we were able to look at sex ratios within litters with good statistical power and did not see a bias in sex ratios within litters. We did not observe a particular sex in the proportion of males with increasing maternal age as we expected from previous studies [17,23]. While we did observe a numerical decline in the proportion of males with increasing litter size among adults and fawns, it was not significant. There was no trend for yearlings and neither age nor litter size explained sex ratios. Reported fetal sex ratios among white-tailed deer are often described as male-biased, however, fetal sex ratios in the literature range from 0.67 to 1.77 (male:female) with a median and mean of 1.1 and 1.2, respectively (Supplementary Table 1).

The artificial selection of hunting pressure can influence sex ratios and age structure of populations [41]. Deer management strategies which typically include hunting often find that populations are female-biased. Our study indicates female-biased populations in Illinois are not a result of reproductive processes. Rather, non-hunting mortality rates among males are typically greater than females [21,29] and male-biased hunting pressure provides an additive effect resulting in female-biased populations.

Birth mass is correlated with survival among cervids [29,42,43] and is important because it can influence the strength and stamina of neonates. Similar to Cothran et al. [28], fetal age was the most important determinant of fetal BMI in our study, and year and geographic location also accounted for some variation in fetal size. Because there can be temporal fluctuations in resources across the landscape, it is not surprising to see some variation in fetal size attributed to such variables. Individual fetal size may be more sensitive than pregnancy rates to variations in resources across the study area and study time period.

In our study, singleton fetuses were small in fawns and yearlings. This was primarily because singleton litters were conceived later based on conception date estimates from crown-rump length. Previous reports indicated that single fetuses were heavier in captive herds [35]. In our study, older females were pregnant with larger fetuses which supports the previous pattern of young females producing smaller fawns compared to older females [8,35].
than adults [45]. Because they are still growing, fawns direct more energy to growth rather than energy storage [45] during breeding season and even a small reduction in caloric intake can inhibit fat deposition [46], potentially suppressing follicle maturation and limiting fawn reproduction [22,37]. This idea is supported by the fact that pregnant fawns and even pregnant yearlings tend to weigh more than their non-pregnant counterparts [37,47].

5. Conclusion

Our study supported several general trends of female reproduction including the increase of pregnancy rates with female age and fawns breeding late in the season compared to older females. We found that fawn reproduction continues to be highly variable and is likely dependent on the interaction between available habitat, deer density, and resource availability which influence fawn growth and fat accretion that, in turn, influence estrus onset. We found balanced fetal sex ratios and evidence that male fetuses are larger than female fetuses. Because our study covered several years, multiple habitat types and deer densities leading to broad scale metrics of female reproduction, future work should focus on studies to better understand the interaction of habitat resources and deer density because they are variable across the landscape.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.theriogenology.2017.02.010.

References