Double Dose of Differentiation

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Introduction

The term “free-martin” was believed to be coined years ago in England. A heifer failed to become pregnant after the summer breeding season and therefore was “free” for fattening. A few months later, she was slaughtered at Martinmas, a fall festival held in honor of St. Martin.[1] This term is now used most commonly to label sterile heifers born co-twin to a bull calf due to the deleterious effects of the male hormones on the development of the female twin’s reproductive tract in utero. This condition is overwhelmingly frequent in heifer calves born co-twin to a bull calf. Though much more common in cattle, this condition does occur in other ruminants (and few other livestock and large animal species) on occasion. Unfortunately for large scale producers, these animals are generally not positively identified as such until the time of puberty. This means months of economic investment in an animal that will rarely produce a (sufficient) return on investment for the farmer. Once positively identified, these animals are commonly culled for slaughter. This may also be the case for smaller ruminants, though these are more likely to just be sold as pets, if not committed to research. Because these animals often present with relatively normal physical exams and external genitalia, a few anomalies of sexual differentiation must be considered. Karyotyping these individuals is highly recommended for a definitive diagnosis. Results will determine how best to manage each situation.

History and Presentation (both typical and atypical)

Freemartin goats most often do not present to the veterinarian until time of puberty. Histories generally consist of the apparently female goat failing to ever come into heat or consistently failing to conceive following the breeding season. Additional complaints may include apparent does developing masculine buck-like characteristics, such as thicker erect hair over the neck, aggression towards people or especially other cycling does, very small

rudimentary teats, and lack of udder development.[2] Clitoromegaly may or may not be appreciated.[2] These individuals are almost always reported to be co-twin to a male or one of a set of heterosexual multiples.[2] The male twin may also present for subfertility.[2] He may also be smaller and appear slightly less masculine than the other bucks in the herd. These animals may be any breed, and horned or polled.

Pathophysiology (include anatomical considerations)

A freemartin results in ruminants when an infertile female is twin to a male.[1] Freemartinsim occurs much more frequently in cattle and thus is better studied and best described in cattle.[1] Normally during early fetal development, the embryonic gonads must undergo an indifferent and a differential stage, beginning around 28 days of gestation.[7] Although genotypic sex is determined at fertilization, all fetuses begin development of gonadal ridges within the intermediate mesoderm alongside the mesonephros, or the primordial tissue of the excretory system.[7] These ridges then form primitive sex cords, which then further develop into an undistinguishable, or indifferent, gonad.[7] Regardless of the predestined sex, primordial tissues of all male and female reproductive structures develop in both sexes.[7] At this point, the fetus has an undifferentiated gonad, a mesonephric (Wolffian) duct system, and a paramesonephric (Mullerian) duct system.[7] Partial to complete regression, remodeling, and the addition of reproductive tissue will then lead to the continued development of the differentiated and respective reproductive structures from these primordial tissues.[7] In the female, the Wolfian ducts degenerate and the genital duct system is derived from the Mullerian ducts.[7] Testosterone from the fetal testes leads to Mullerian duct degeneration, and thus a male reproductive tract forms.[7]

In the freemartin, the placentas of each fetus will then fuse together around 30-50 days gestation, resulting in shared blood for the remainder of the pregnancy.[1] Because this fusion occurs before gonadal differentiation, occurring around 40-50 days gestation, each twin calf is an XX/XY chimera.[1] The testis determining factor, or TDF, also referred to as the sex-determining region Y factor, or SRY gene, is found on the Y chromosome carried by the male twin.[6] The presence of this gene then arrests the development of the female reproductive tract, thus influencing the freemartin's gonads to begin development towards testis.[1] Each freemartin will vary in the degree of differentiation, though many freemartin gonads remain undifferentiated, resulting in an ovotestes.[1] The shared blood supply during the development allows for testosterone and anti-mullerian hormone (AMH) from the Sertoli cells of the developing testes of the male twin to directly disrupt the development of the freemartin's female reproductive tract.[1,5] The freemartin will then fail to develop mullerian duct derivatives, such as the cranial vagina, cervix, uterus, or uterine tubes.[1,5] External genitalia generally appears fairly normal to slightly underdeveloped.[1] In cattle, the yearling freemartin will fail to exhibit estrus and the udder and teats will remain rudimentary in size and development.[1] The freemartin will appear slightly more masculine, externally resembling a steer, though with a vulva.[1] The male twin can develop into a fertile adult, but has a higher chance of failing a breeding soundness examination than bulls possessing 60, XY genotypes.[1] These individuals are more likely to become steers.[1]

Freemartinism is a common source of chimerism in ruminant twins.[1] These individuals contain cell lines of two different embryonic sources.[1] This occurs after the natural fusion of blastocysts in utero.[1] However, the rare possibility of blastomere fusion following double ovulation and fertilization in the horse has been suggested, resulting in 64, XX/ 64, XY and 63, XO/64, XY genotypes.[1]

Birth to multiples is more common in goats, but vascular anastomosis is not.[1] Though rare, shared circulation is more likely to occur after the critical period of sexual differentiation in goats.[1] Caprine freemartins result when a doeling is twin to a buckling or is one of heterosexual multiples.[2] Breeders must keep in mind that freemartins may still be born as a singlet following the death of a twin in utero.[2]

Differential Diagnoses

Sexual ambiguity is relatively common among domestic goats. Though, varying degrees of each condition often complicate simple diagnosis. Intersex conditions are more frequently identified, though present in different forms. Intersex conditions imply a disagreement of chromosomal sex with gonadal sex and/or phenotypic sex, further implying pathology of the sex-determining genes.[5] These conditions are believed to result from incomplete masculinization of the duct system or the external genitalia by the fetal testes, thus the goat is left with parts of the mesonephric and paramesonephric ducts.[1] These goats are further classified as true or pseudohermaphrodites.[2] Intersex conditions may occur in all goat breeds, though prevalence varies among breeds and secondary to breeding management of specific lines. These intersex conditions do however occur more among polled dairy goats, such as Saanens, Toggenburgs, and Alpines.[1]

Male pseudohermaphrodites occur more often in dairy goat breeds over wool breeds.[2] These are even more common in those selected for twinning and/or the polled (lack of horns) gene.[2] One study among US dairy goat breeds found 11% of Saanen and 6% of Toggenburgs to be intersexes compared to less than 2% prevalence among European dairy goats bred to native Indian goat breeds.[2] As much as 20% of the offspring of polled-to-polled matings may exhibit intersexuality.[2] The recessive polled gene, P, is dominant over the horn gene, p, in heterozygous

kids.[2] The genes for hermaphroditism and sex reversal are thought to be linked to the polled gene.[2] Polled intersexes are karyotypically female (XXPP), while fertile polled does are XXPp.[2] The exact mechanism of sex reversal in these females is unknown, though one theory proposes the possibility of translocation of the SRY factor to the X chromosome or even an autosome.[2] Another theory proposes the ability of the P gene, or a closely neighboring segment, to possess the ability to act as a Y chromosome and initiate testicular formation in an XX karyotype.[2] Male pseudohermaphrodites more commonly appear more 'female-like' at birth in regards to appearance of external genitalia.[2] However as these animals age, they become more masculine in appearance. They tend to grow larger, neck hair grows in thicker and more erect, and external genitalia may now include a short penis or bulbous clitoris that becomes visibly enlarged by the time of puberty.[2] Hypospadias, or the dislocation of the urethral orifice to the ventral aspect of the penis, may be represented by narrow slits along the ventral midline following the failed fusion of the urethral folds.[2] It has also been documented for these folds to spill urine into a caudal pouch, resembling a scrotal sac in a relatively normal location.[2] Anogenital distance may range from 3-33cm in length among caprine intersexes dependent upon individual gonadal development.[2] By rule of thumb, individuals with an anogenital distance of 3-6cm tend to have intra-abdominal gonads, consisting of poorly developed epididymis, vas deferens, seminal vesicles, and moderately developed mullerian duct derivatives, such as oviducts, uterine horns, and uterine body.[2] Individuals with longer anogenital distances tend to be exhibit more masculine genitalia, both internally and externally.[2] This may include scrotal or inguinal gonads, an enlarged clitoris or an actual sheathed penis, and poorly developed uterine horns and body.[2] These individuals tend to act more masculine with age, including head butting, aggression towards other goats and humans, urine dribbling or forward directed urination between the legs

with outstretched legs and a concave back, development of the characteristic odor of a buck, and increased male libido in the presence of a normal doe in estrus.[2] Male pseudohermaphrodites generally have intra-abdominal testes residing in the location of normal ovaries to fully descended testes.[2] Partially descended testicles may become trapped in the inguinal area and become layered with prominent fat pads that are later mistaken for udder development at puberty.[2] It is less commonly possible for a uterine horn to become wrapped around or attached to a descending testicle and thus herniates into the scrotum.[2] Other cases may include posterior parts of horns connecting to a shortened uterine body that either opens into a cervix or is closely associated with the vas deferens.[2] These uterine horns are most commonly distended with mucus into adulthood, forming bilateral hysteroceles.[2] This trait, combined with scrotal herniation, is commonly mistaken for severe orchitis at puberty.[2] Male pseudohermaphrodites possessing intra-abdominal testes are generally much smaller testicles than those of a normal buck of the same age.[2] Seminiferous tubules remain narrow and immature in size, irregularly outlined, and lined only by Sertoli cells at puberty.[2] As the animal ages, the tubular basement membrane is thick and hyalinized while the seminiferous tubules appear atrophied with abundant interstitium comprised of varying degrees of Leydig and fibroblastic cells.[2] In most cases, the most abundant steroid produced by the caprine intersexes is testosterone, though at lower levels than normal bucks.[2] Due to the absence of testicular germ cells, male pseudohermaphrodites are always sterile.[2] Male pseudohermaphrodites are the most common intersexes seen in dairy goats specifically selected for twinning and multiples.[2]

The less common true hermaphrodite is the result of two cell lines of differing karyotypes arising from the same zygote.[1] These animals have mixed gonadal dysgenesis with bilateral intraabdominal ovotestes or an ovotestes accompanied with a contralateral testis or ovary.[1, 2]

Ovarian tissue is still hormonally functional and follicles can be found at various stages.[2] The seminiferous tubules found within the ovotestes, lack male germ cells and are thus nonfunctional.[2] These individuals often display gonadal hyperplasia, tumors, or both.[2] These animals are generally more phenotypically female, internally and externally.[2] True hermaphrodites are most often total chimeras, 60/XX, 60/XY.[2] This particular chimerism results from comingling of cells from male and female embryos in early embryogenesis or through fertilization of the second polar body and the ootid by spermatozoa carrying different sex chromosomes.[2] This accidental fertilization anomaly can happen in any and all breeds of goat.[2]

Diagnostic Approach/Considerations

As high as 92% of phenotypic heifers born co-twin to a male are believed to be freemartins.[1] These animals generally fail to ever exhibit estrus, and rectal palpation of breeding age heifers reveals significant reproductive tract abnormalities. These abnormalities range from hypoplasia to complete aplasia of the reproductive tract beyond the caudal vagina.[1] Rectal palpation may not be possible in smaller animals, but a vaginal exam with a glass test tube or speculum may be performed to confirm a shorter, likely blind-ended, vaginal pouch.[1] Freemartin cattle are generally found to have 6-7cm long vaginas, as compared to a normal heifer with a vagina literally double this length.[1] Male twins are generally normal, though may exhibit subfertility and potential cryptorchidism.[3]

Options for definitive diagnosis are few. Abdominal exploratory surgery is a potential option, though highly invasive and not economical. However, this remains an option should the animal require abdominal surgery for another reason. Transabdominal ultrasound may be considered in an effort to identify testes, ovaries, or other tubular reproductive structures, though is more often subjective and unrewarding.

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The most definitive diagnosis includes karyotyping to confirm blood cell chimerism. It must be kept in mind that a varying amount of male cells are present in freemartins. [1] Freemartinism suspected in singlet kids requires confirmation through karyotyping. [2] When karyotyping these individuals, one must take great care in evaluating an adequate number of cells as the proportion of male cells in leukocyte culture could be as low as 1%.[2] Solid tissues of caprine freemartins contain exclusively female cells, though blood contains female and male cells. [2] One study used the SRY gene as a marker to confirm the absence or presence of the Y chromosome among XY heterochromosomes in three doelings co-twinned to males. [4] Cell chimaerism was diagnosed with SRY gene PCR and confirmed with positive identification within T lymphocytes using further cytogenetic analysis. [4] Chromosomal preparations were then able to be stained and a metaphase examination performed. [4] These diagnostics were able to positively identify this chromosomal abnormality, but furthermore excluded the theory of translocation of SRY-containing Y chromosome onto another autosome or the X chromosome as previously suggested. [4] The cytogenetic examinations were also able to quantitatively estimate the percentage of XY chromosomes originating from the male twin. [4]

Treatment and Management Options

Management options regarding the rate of occurrence of freemartinism is neither straightforward nor rewarding at this time. Intersexes and freemartins fail to produce sperm at

any point during the year and lack the strong odor of a normal buck, thus breeding by these individuals is not of concern.[2] Although freemartins are unable to reproduce, it is best to prevent attempts of copulation. Most freemartins possess a shortened vaginal pouch and vaginal penetration by a buck is often painful and thus should be avoided.

Intersex conditions are believed to be caused by the recessive gene linked for polledness in dairy goats. Although there is no treatment for this genetic anomaly, breeders may take more caution to prevent mating of two polled goats.[1] While this may dramatically decrease the chances of intersex offspring, breeders must keep in mind that intersex conditions may still result from the breeding of horned goats.[1] Prevalence of intersexuality in feral, horned goats is however extremely low.[1]

Expected Outcome and Prognosis

Freemartin goats may still lead a perfectly healthy and otherwise long, normal life. This condition is benign and nonproblematic when the animal is only intended to be kept as a pet. However in the case that the animal is to be kept in an industry setting, these genetic anomalies are undesired as they do not contribute toward production. These animals are sterile and unable to produce offspring, and thus unable to lactate. These conditions are often never identified until failure to cycle or subfertile bucks are identifed at puberty. Thus these animals consume months of pointless economic investment until finally culled upon diagnosis. These animals may then be sold as pets or sent to slaughter in order to compensate for lost investment thus far.

Other Pertinent Information

Freemartinism is less common among sheep, goats, and even more rarely observed in pigs. This is mostly due to the higher incidence of naturally occurring multiples in these species.

Freemartinism occurs in 1% of ewe lamb heterosexual twins, over 90% of heifer calves born co-twin to a bull calf, and comprise 6% of all intersex conditions diagnosed in goats.[1]

However, sheep freemartinism is much less rare in twins or triplets, than is seen with quadruplets or even quintuplets.[1] Sheep freemartins are more likely to develop much more masculine features than seen in cattle which may be associated with the development of gonads resembling cryptorchid testes.[1] These freemartins may also develop epididymides, vasa deferentia, vesicular glands, and even cremaster muscles.[1]

Conclusion

Goats are more likely to give birth to multiples than single kids. This is even more likely in some breeds than others after long lines of selection for birthing multiples. With each additional multiple per pregnancy, chances increase for abnormalities to arise during fetal development. These adverse chances are further exacerbated when certain additional traits are selected for, particularly the polled gene in dairy goat breeds. Abnormalities of sexual determination and differentiation have long been a concern of high producing goat breeders for years. Breeders most often select for multiples to increase numbers of kids born and risk higher chances of intersexuality when also selecting for polled traits in dairy goats. Breeders are encouraged to seek further diagnostics, particularly karyotyping to differentiate between intersexuality and freemartinism in suspect kids. When dealing with higher rates of intersex kid crops, the breeder must take care to avoid polled-to-polled matings in future pairing. Meanwhile, caprine freemartins are identified only 6% of intersexes.[1] No breeding management changes are recommended at this time as freemartins may happen in any breed of goat, polled or horned. Few sources subjectively recommend culling any prolific lines that have produced freemartins.

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